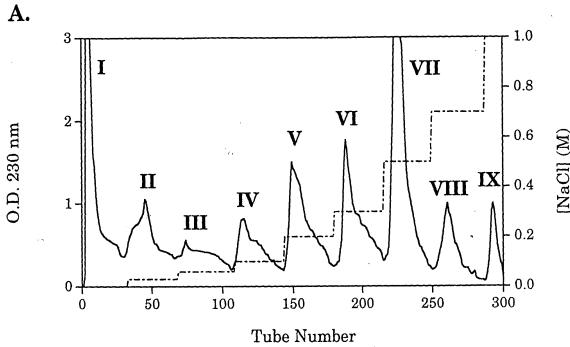
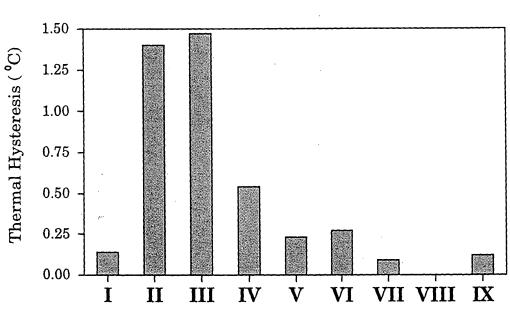
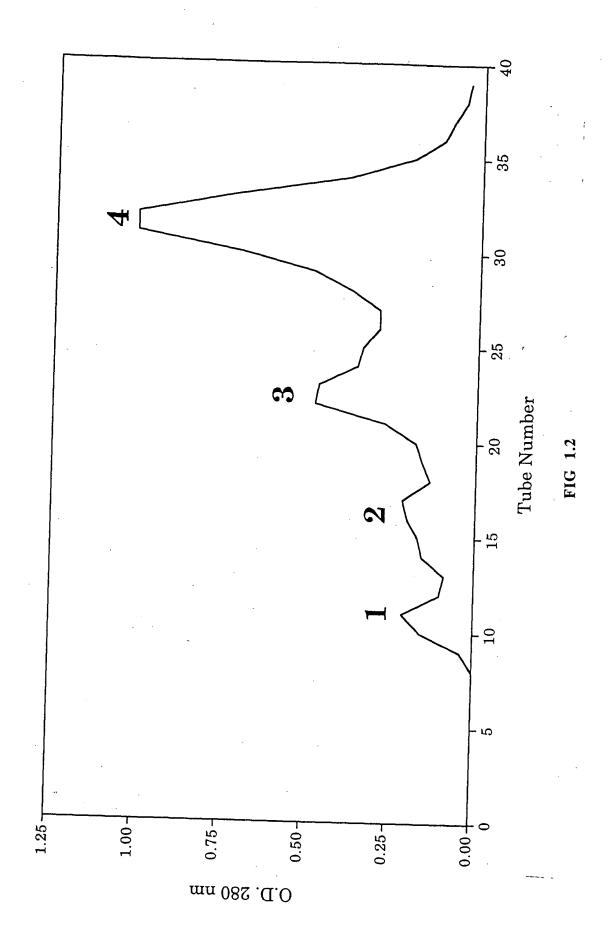


В.





Ion Exchange Peak at 50 mg/ml FIG 1.1



Tm-12.86



12.5 25



FIG 1.3

FIG 1.4

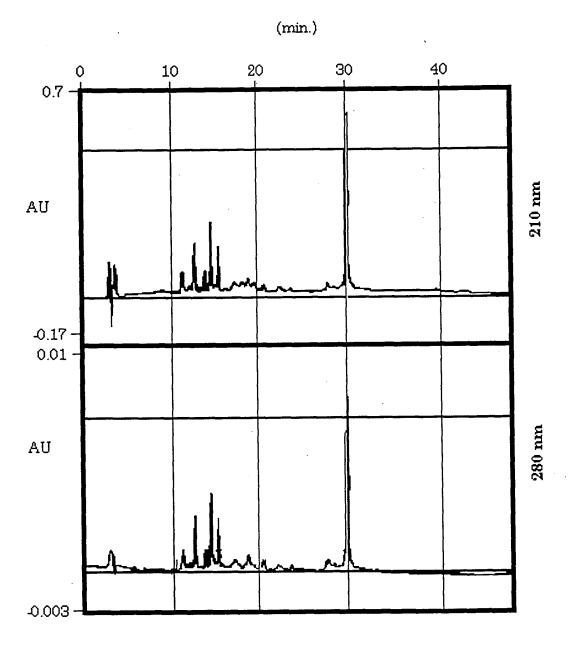


FIG 1.5

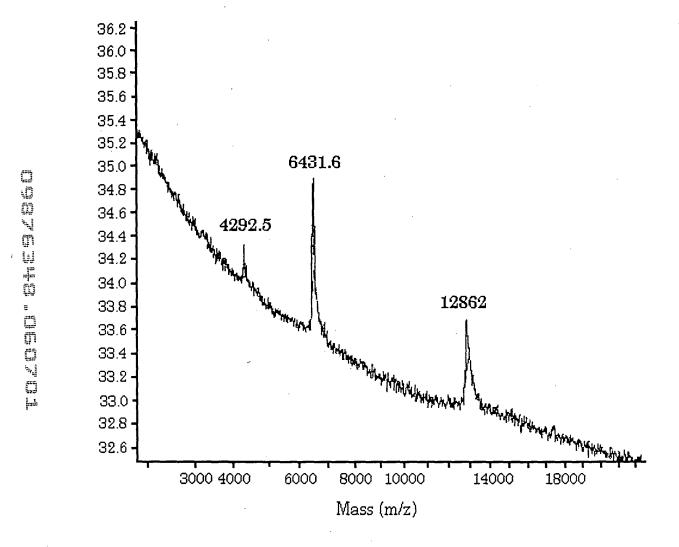


FIG 1.6

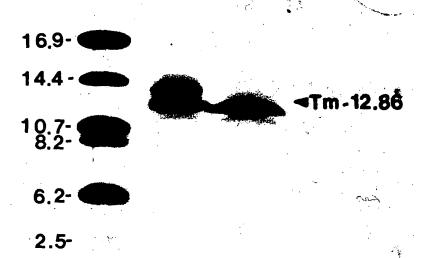
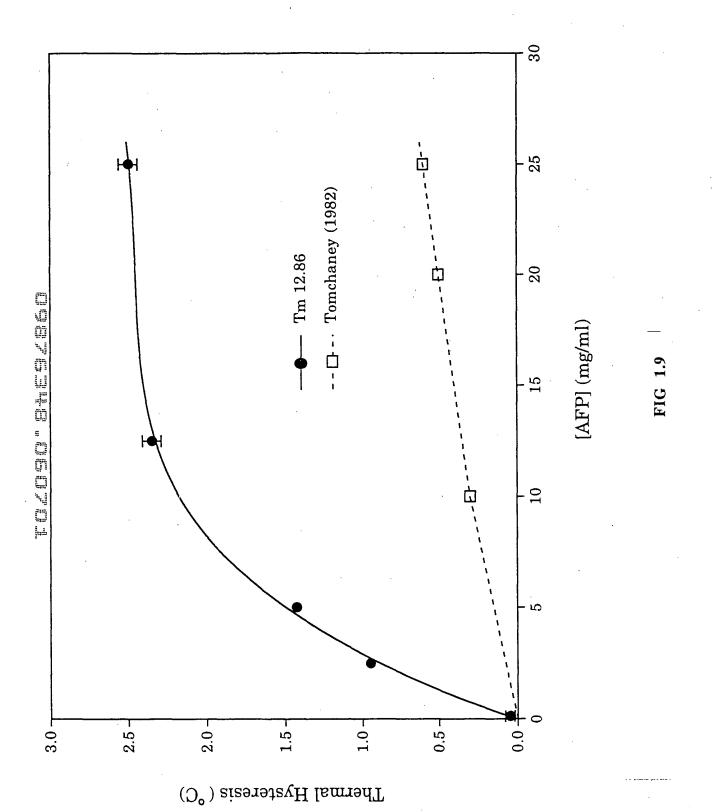


FIG 1.7

FIG. 1.8



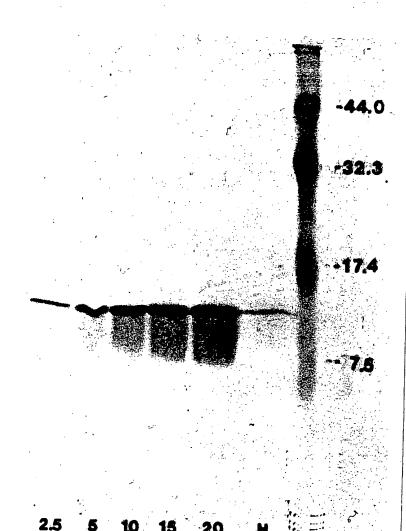
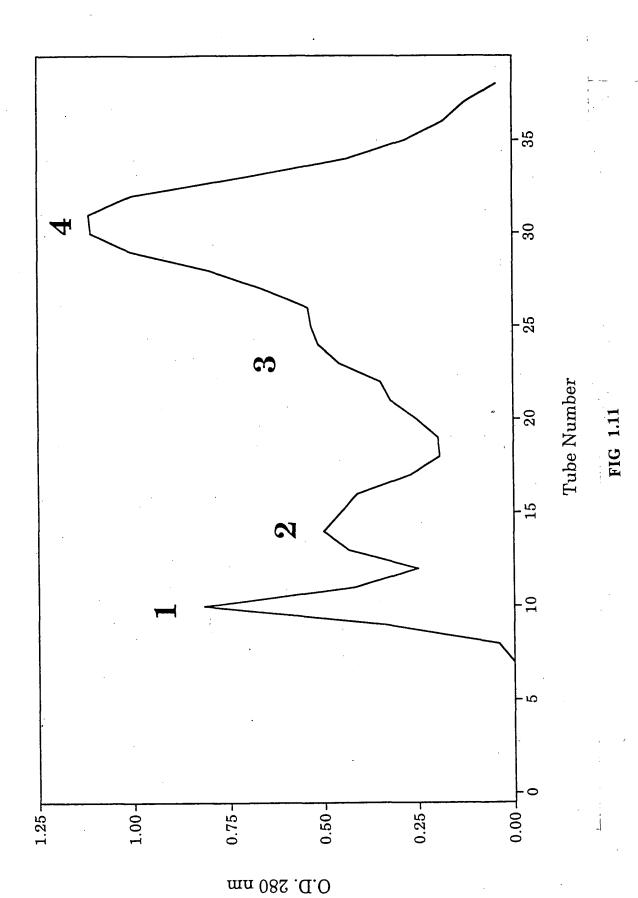


FIG 1.10



Absorbance

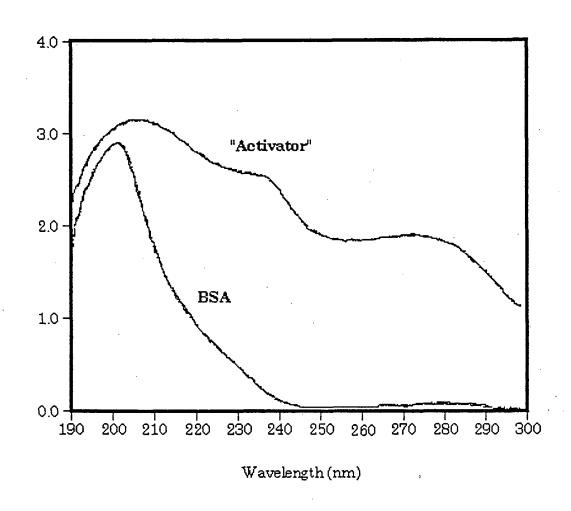


FIG 1.13

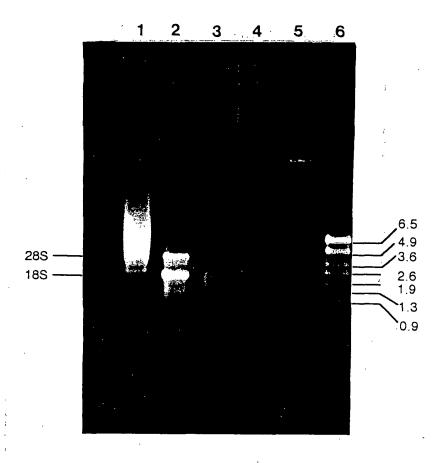


FIG 2.0

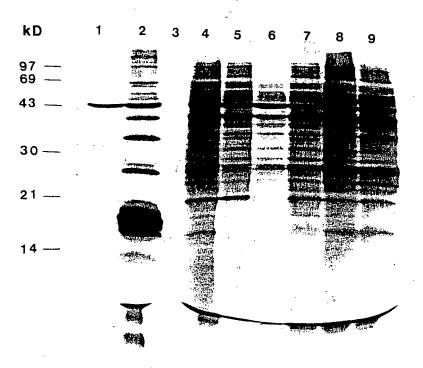


FIG 2.1

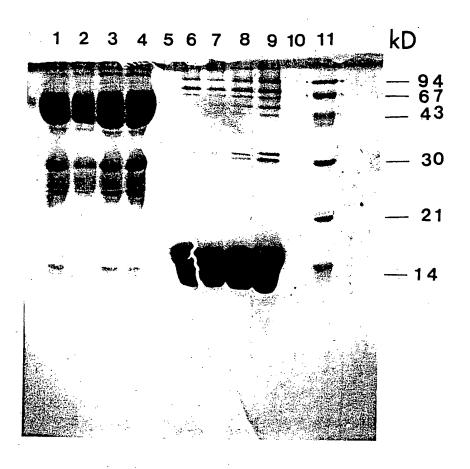


FIG 2.2

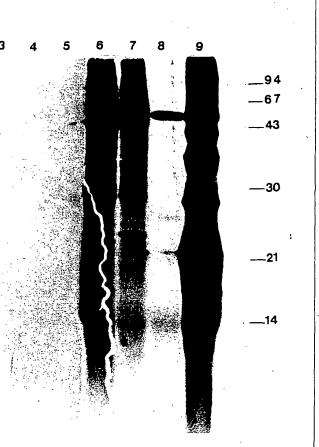


FIG 2.3

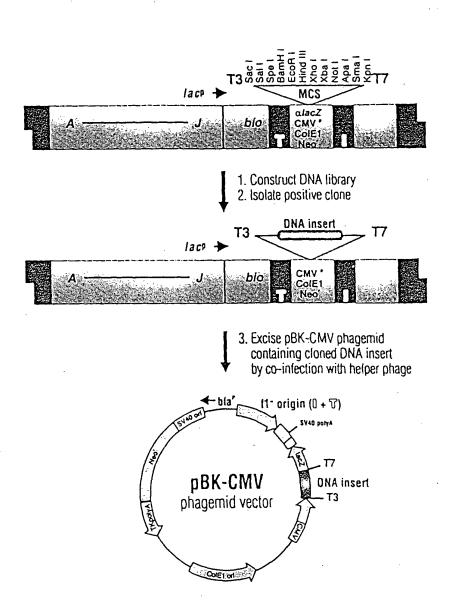


FIG. 2.4 a

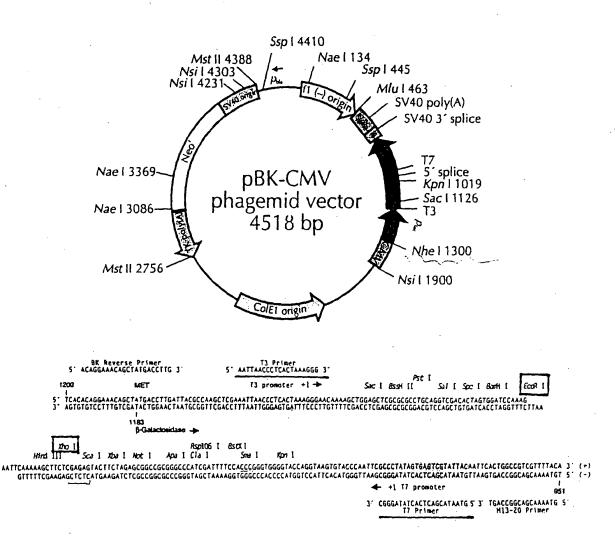


FIG. 2.4 b

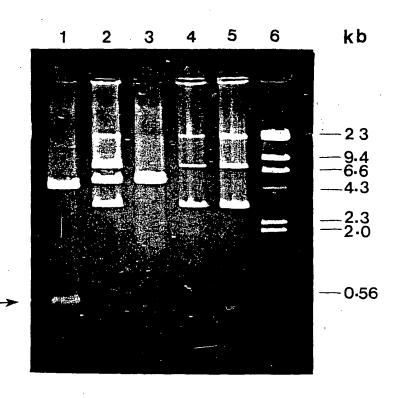


FIG 2.5

DNA sequence of Tm 13.17 cDNA clone

	1	3		F	2															
	i	a		C	>															
	1	n		C)															
	Į	∄ ं		F	Ł															
	:	Γ.		1	:															
1	AGT	GA?	rcca	AAC	IAA	TCC	GC.	CGA	GAC	TAC	TAP	ADA	GAA	GTT	GCI	CTC	TTG	TCT	'AAT	CT
												M	K	L	L	C	C	<u> </u>	I	<u>_S</u>
61	CCCI																			
	L	I	L	L	V	T	V	_الا	_ <u>A</u>	lacksquare	T	E	A	Q	I	Ε	ĸ	L	N	K
										•										
121	AGAI					_	_													
	I	S	K	K	C	Q	N	E	S	G	V	S	Q	E	I	Ī	T	K	A	R
							*=													
181	GCAA																			
	N	G	D	W	E	D	D	P	K	L	K	R	Q	V	F	С	V	A	R	N
					·															
241	ACGC																			
	A	G	L	A	T	E	S	G	E	V	V	. V	·D	V	L	R	Ε	K	V	R
	<u> </u>																		. <u>.</u> .	
301	GGAA				~															
	K	V	T	D.	N	D	Ε	E	T	E	K	I	I	N	K	C .	A	V	K	R
						_			_			_		٠	<u></u>					
361	GAGA														-					
	·D	T	V	E	E	T	V	F	N	T	F	K	С	V	M	K	N	, K	P	K
									· 		<u>-</u>									
421	AGTT					TTG	AAC	CAC	CAC	GAC	TAG	TAG	ATG	GTT	CAA	ATG	GTG	TGC	TTT	AC
	F	S	P	V	D	*													.,	
																			X	
																			h	
							•												0	

537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTTCCACCC

cleavage site

MK, LLCCLISLICCV, TVQA,

n-region (basic) h-region (central hydrophobic) c-region (more polar)

FIG 2.6b

A. Mature Tm 13.17 amino acid residure

- 1 LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR
- 51 NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN
- 101 TFKCVMKNKP KFSPVD

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala B = Asx C = Cys D = Asp E = Glu F = Phe G = Gly H = His	6 0 4 8 13 4 4	5.172 0.000 3.448 6.897 11.207 3.448 3.448
I = Ile	6	5.172
K = Lys	16	13.793
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	. 3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V =Val W = Trp	14 1 0	12.069 0.862 0.000
Y = Tyr Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

FIG 2.6c

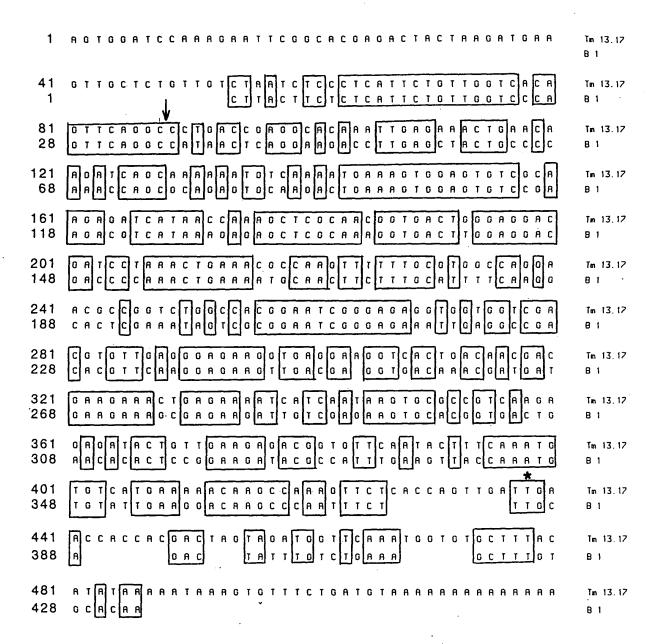


FIG 2.7

Tm 13.17	3	EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA	52
AFP-3	1	ETPREKLKQHSDACKAESGVSEESLNKVRNREEVDDPKLKEHAFCILKRA S	50
Tm 13.17	53	GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF	102
AFP-3	51	GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF	100
Tm 13.17	103	KCVMKNKP 110	
AFP-3	101	KCVHDNRS 108	

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

FIG 2.8

Ç.

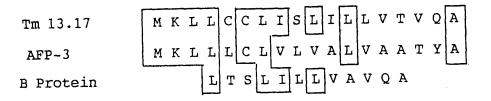


FIG 2.9

Tm	13.17	NH2-L T	E	A	Q	I	Ε	K	L	N	K	I	S	K	K	С	Q	И	E	
Tm	12.86	NH2-L T	D	Ε	Q	I	Q	K	R	N	K	I	S	K	E	?	Q	Q	٧	

FIG 2.10

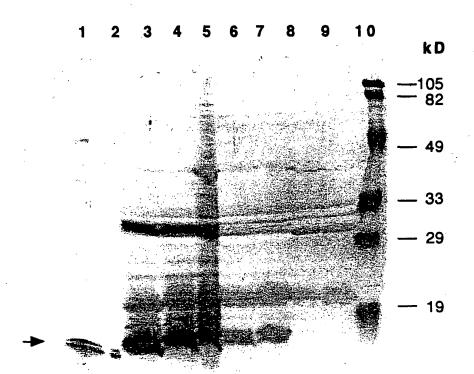


FIG 2.11

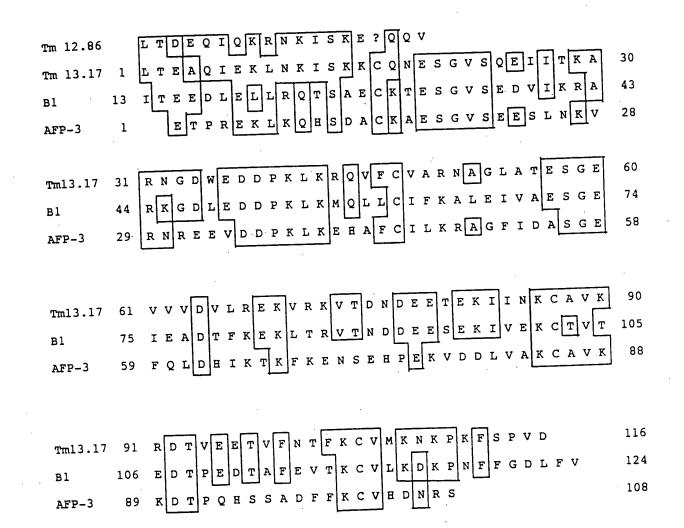
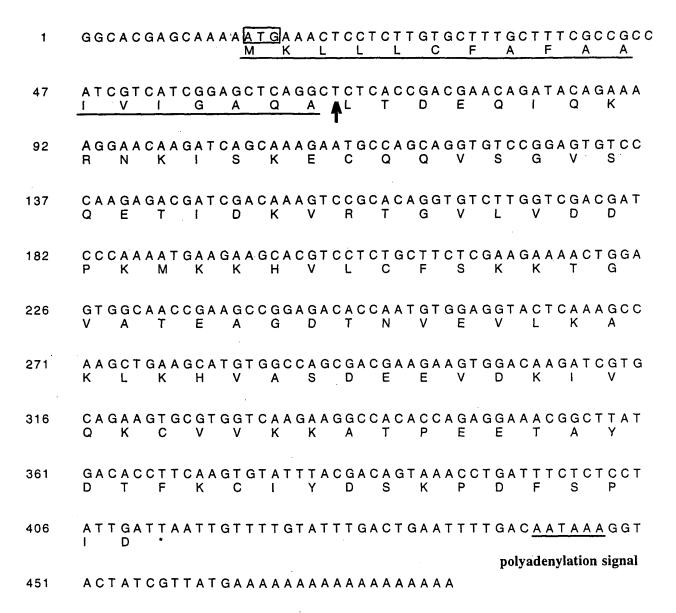


FIG 2.12

	1	GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGC
	47	ATCGTCATCGGAGCTCACCGACGAACAGATACAGAAA IVIGAQAALT DEQIQK
	92	AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCCRNKISKECQVSGVS
, 100 min	137	CAAGAGACGATCGACAAGTCCGCACAGGTGTCTTGGTCGATGAT Q E T I D K V R T G V L V D D
	182	CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA P K M K K H V L C F S K K T G
	226	GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC V A T E A G D T N V E V L K A
	271	AAGCTGAAGCATGTGGCCAGCGACGAAGATCGTG K L K H V A S D E E V D K I V
	316	CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT Q K C V V K K A T P E E T A Y
	361	GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCCT D T F K C I Y D S K P D F S P
	406	ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
	451	polyadenylation signal ACTATCGTTATGTAAAAAAAAAAAAAAAAAAAAAAAAAA

poly (A) tail



poly (A) tail

FIG 3.1

start

```
G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C G
2-2
     G G C A C G A G C A AAAAT GAAACT CCT CTT GT G CTTT G C T
2-3
     T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G
     T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G
     A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A
2-2
2-3
     A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A
     A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C
2-2
     A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C
2-3
     G A C A A A G T C C G C A C A G G T G T C T T G G T C G A T G A T C C C A
2-2
     G A C A A A G T C C G C A C A G G T G T C T T G G T C G A C G A T C C C A
     A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A C
     A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A C
     2-2
     2-3
     G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G
2-2
     G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G
2-2
     A A G A G T G G A CAAGAT CGT GCAGAAGT GCGT GGT CAA
     A A G A A G T G G A CAAGATCGTGCAGAAGTGCGTGGTCAA
2-3
2-2
     G A A G G C C A C A C C A G A G G A A C G G C T T A T G A C A C C T T C
2-3
     A A G T G T A T T T A C G A C A G C A A A C C T G A T T T C T C T C C T A
2-2
     A A G T G T A T T T A C G A C A GÍT A A A C C T G A T T T C T C T C C T A
     T T G A T T A A T T GTTTTGTATTTGACTGAATTTTGACAA
2-2
     T T G A T T A A T T GTTTTGTATTTGACTGAATTTTGACAA
     TAAAGGTAATATCGTTATGITAAAAA
2-2
     T A A A G G T A C I T A T C G T T A T G A A A A A
2-3
```

Predicted Amino Acid Composition of 2-2 and 2-3

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis									
	Number	% by	% by						
Amino Acid(s)	count	weight	frequency						
Charged (RKHYCDE)	48	47.19	41.74						
Acidic (DE)	20	18.90	17.39						
Basic (KR)	20	20.40	17.39						
Polar (NCQSTY)	30	25.35	26.09						
Hydrophobic (AILFWV)	34	27.26	29.57						
A Ala	6	3.32	5.22						
C Cys	4	3.21	3.48						
D Asp	11	9.86	9.57						
E Glu	9	9.05	7.83						
F Phe	3	3.44	2.61						
G Gly	4	1.78	3.48						
H His	2	2.14	1.74						
l lie	6	5.29	5.22						
KLys	. 18	17.97	15.65						
L Leu	5	4.41	4.35						
M Met	1	1.02	0.87						
N Asn ·	2	1.78	1.74						
P Pro	4	3.02	3.48						
Q Gin	6	5.98	5.22						
R Arg	2	2.43	1.74						
S Ser	7	4.75	6.09						
TThr	j 9	7.08	7.83						
∨ Val	14	10.80	12.17						
W Trp	0	0.00	0.00						
Y Tyr	2	2.54	1.74						
B Asx	0	0.00	0.00						
Z Glx	0	0.00	0.00						
X Xxx	0	0.00	0.00						
. Ter	0	0.00	0.00						

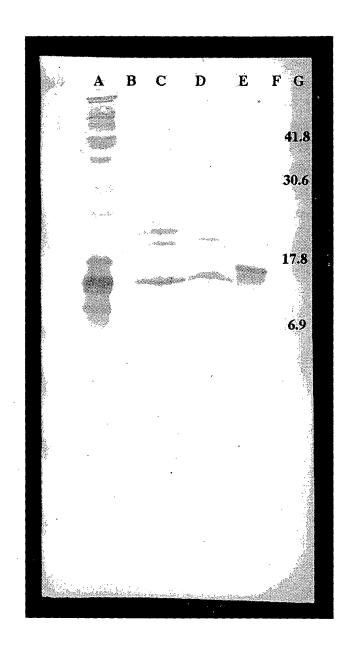


FIG 3.4



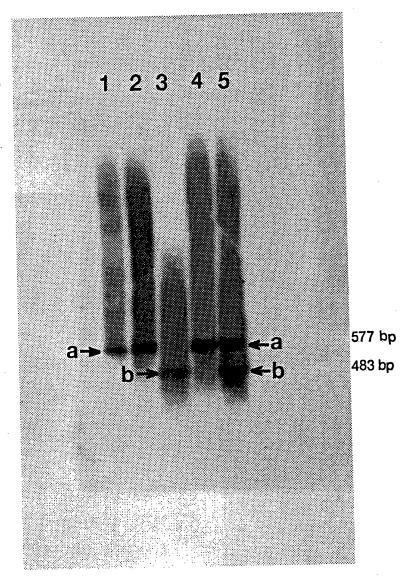


FIG 4.0

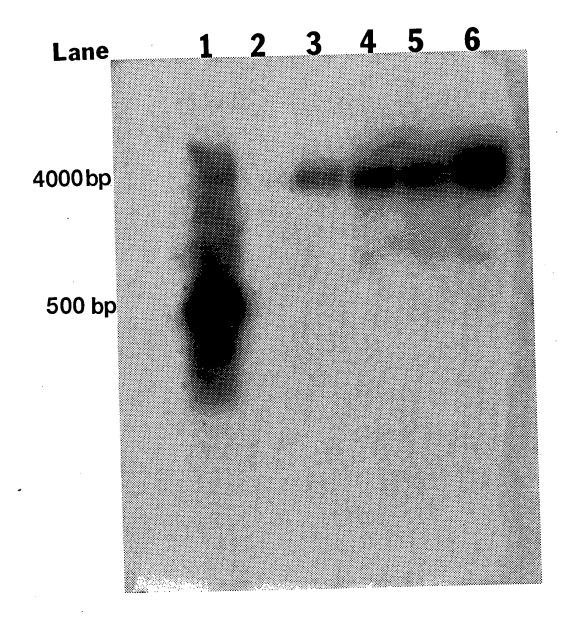


FIG 4.1

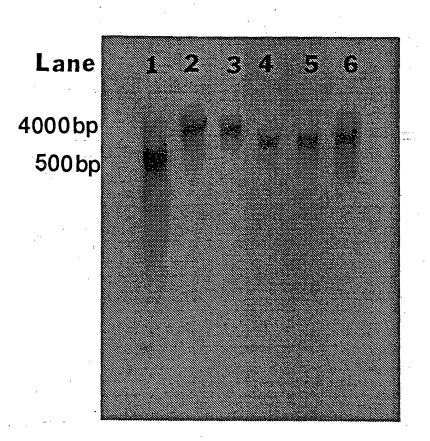


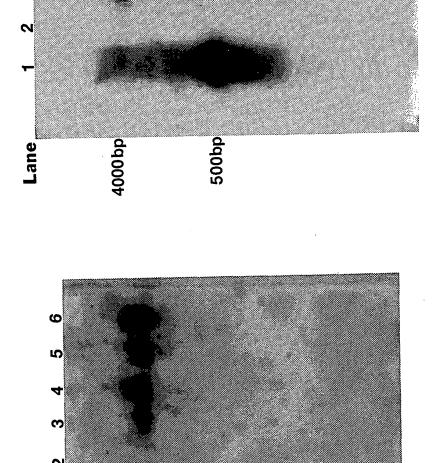
FIG 4.2

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F1G 4.3

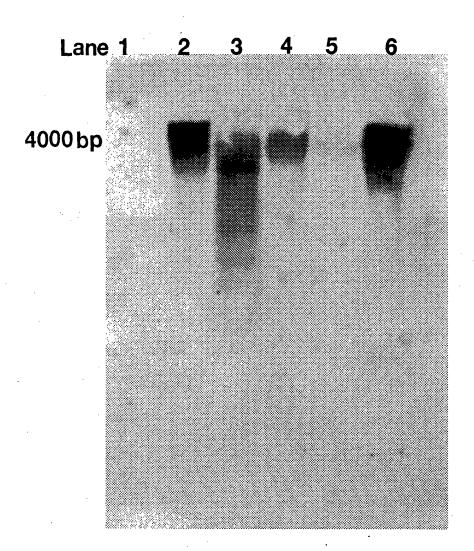


FIG 4.4

Lane 1 2 3 4 5

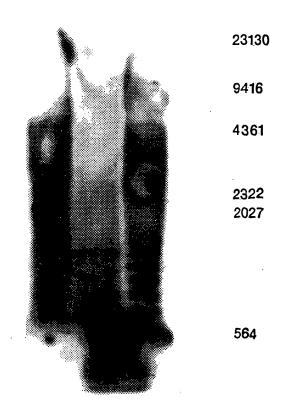


FIG 4.5

Tm 13.17 cDNA

1	AGTO	GAI	CCF	AAC	LAAI	TCC	GCA	ACGA	AGAC	CTAC	TAA	TAD	GAA	GTT	GCT	CTG	TTG	TCT	AAT	CT
												M	ĸ	L	L	С	C	L	I.	<u>s</u>
61	CCCI	'CAT	TCI	'GTI	'GGI	'CAC	'AGT	TCA	\GGC	CCI	'GAC	CGA	.GGC	ACA	AAT	TGA	.GAA	ACT	GAA	CA
	_		L		V	T						E ird P			I	E	K	L.		ĸ
121	AGAI	'CAC	CAA	AAA	ATC	TCA									GAT	CAT	AAC	CAA	AGC	TC
		S		ĸ	С		N	E		_	٧			E	,	Ī	T	K		R
181	GCAA	.CGG	TGA	.CTG	GGA	.GGA	.CGA	TCC	TAA	ACI	ĠA.	ACG	CCA	AGT	TTT	TTG	CGT	GGC	CAG	ga
	N	G	D	W	E	D	D	P	ĸ	L	K	R	Q	V	F	С	Ÿ	A	R	N
241	ACGC	.CGG	TCT	GGC	CAC	GGA	ATC	:GGC	AGA	.GGI	'GGT	'GGT	CGA	.CGT	GTT	GAG	GGA	.GA.A	.GGT	GA
	A	G	L	A	T	E	S	G	Ε	V	V	. V	D	V	L	R	E	ĸ	V	R
301	GGAA	.GGT	CAC	TGA	.CAA	.CGA	.CGA	AGA	AAC	TGA	GAA	AAT	CAT	CAA	TAA	GTG	CGC	CGT	CAR	GA.
	K Re	•	T se l	D Prim	N ier	D	E	E	.T	Ε	ĸ	I	I	И	ĸ	С	A	V	٠K	R
361	GAGA	TAC	TGT	T.GA	AGA	GAC	GGT	GTT	CAA	TAC	TTT	CAA	ATG	TGT	CAT	GAA	AAA	CAA	.GCC	ĄĄ
	D	T	V	E	E	T	V	F	N	T	F	ĸ	С	V	М	K	И	ĸ	P	K
421	AGTT	CTC	ACC	AGT	TGA	TTG	AAC	CAC	CAC	GAC	TAG	TAG	ATG	GTT	CAA	ATG	GTG	TGC	TTT	AC
	·F	S	P	V	D	*														

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2-2	LTDEQIQKRNKI SKECQQVSGVSQETI DKVRTGVLV
Tm 13.17	LTEAQIEKLNKI SKKCQNESGVSQEI I TKARNGDWE
B2	LTEEDLQLLRQTSAECKTESGASEAVI KKARKGDLE
AFP-3	ETPREKLKQHSDACKAESGVSEESLNKVRNREEV
2-2	DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKH
Tm 13.17	DDPKLKRQVFCVARNAGLATESGEVVVDVLREKVRK
B2	DDPKLKMQLLCIFKALEIVAESGEIEADTFKEKLTR
AFP-3	DDPKLKEHAFCILKRAGFIDASGEFQLDHIKTKFKE
	Reverse Primer
2-2	VAS DEEVDKI VQKCVVKKATPEET AYDTFKCI YDS
Tm 13.17	VTDNDEETEKI I NKCAVKRDTVEET VFNTFKCVMKN
B2	VTNDDEESEKI VEKCTVTEDTPEDT AFEVTKCVLKD
AFP-3	NSEHPEKVDDLVAKCAVKKDTPQHSSADFFKCVHDN

percent % composition

^		percer	11 % C	omposit	ion	,	
C	Primer	· A	С	G	Т	MeltingTemperature (OC)	
_							
	Forward	28 6	14.3	42.9	14.3	44.0	
		05.0	04.0	0.0	07.5	44.0	
	Reverse	25.0	31.3	6.3	37.5	44.0	

FIG 4.6

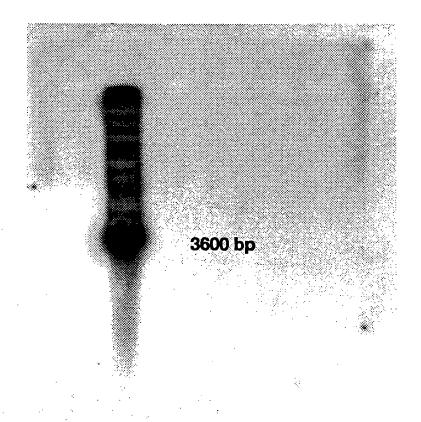


FIG 4.7

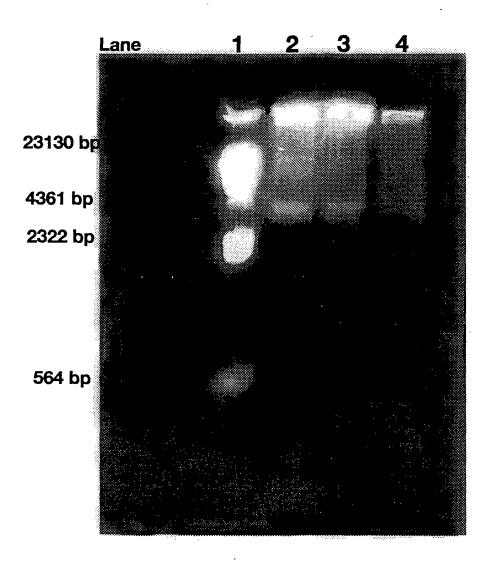


FIG 4.8

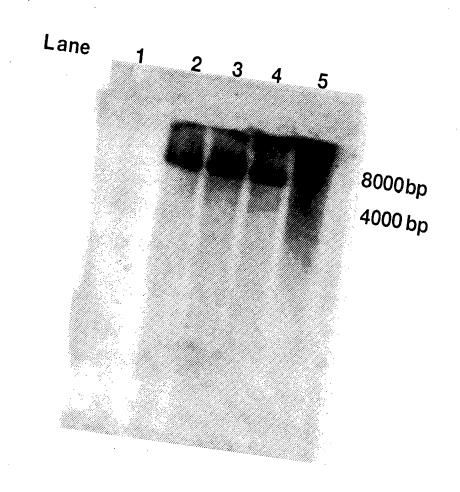


FIG 4.9

1	G	G	С	Α	С	G	A	G	C A	Α /	4 A	A A	A	_	G	A K		A	C L	T (C T		٠.			T C			T T		-	с —		T F			G (C C	G A	сс -	
47	A	T —	С	G V	T	С	A I	T		G (G /	A (Т	Ca					T (rc	A T			G D	Α!		G A E	A A	C			A	Т		C A	A (G A K		Α	
92	A R			A N			A K		G ,	A 7	r		A G	i C	A			G	Α		T (3 (CQ			G T V	ΓG	i T S			G G			G ·	T	G T S		С	
137	CQ	Α		G E	Α	-	A T	C	G	А ⁻	Γ(C (C	A			G V	Т	_	C (R	G (A T	_		G G	G '		G T V	ГС	T L	Т		G V	Т		G. D	A	C G		T	
182	C P	С		A K			A M						Α A (C			G V	Т	C	C ⁻ L	rc	C	G	С	T	Т	c ;	T (S	C G							A '	C.	T G		Α	
226	G V		G	G A			A T		С		Α /		3 C								A (A N			G V			G /	۹ و	G V			C			A. K		A C		С	
271	A K			C	T		A K						3 T /					A S		C	G / D	Α (C G	A	Α	G E	Α	G (G ⁻ V	ГС	G D	i A		A K		G	A T	Τ	C G	ìΤ	G	
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poly (A) tail

FIG. 4.10 a

Analysis	Whole Protein
Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Whole Protein Composition Analysis

Whole Protein Compositi	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHY.CDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AILFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lle	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
·M Met	1	1.02	0.87
N Asn	· 2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.99	5.22
R Arg	2 7	2.43	1.74
S Ser		4.75	6.09
T Thr	9	7.09	7.83
V Val	15	11.58	13.04
· W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Predicted Amino Acid

Composition of 3-4

FIG. 4.10 b

451

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226	G V	Т	G	G A	С	Α	A T	С	С	G E	Α	Α	G A	С	С	G G	G	Α	G D	A	C :	A (c	A C	A I	Τ	G V	Т	G	G / E	4 C	à G V		Α				A K	Α.	Α (G C	С	
271	A K									C H			G V	Т		G A			A. S			G A	A (à A E		G E			G V	ΓΘ	à G D			A K		G	A I	т (3 T /	G	
316	CQ			A K	Α	G	T C	G		G V		G	G V	Т	C	A K	Α		A K			G (A				; А	C P	С	Α	G / E	۹ (G E	iΑ		A T	С		G A	C ·		Γ A /	ΛT	
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poly (A) tail

FIG. 4.11 a

ACTATCGTTATGAAAAAAAAAAAAAAA



Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole	Protein	Composition	Analysis

	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
l lle	6	5.28	5.22
K Lys	17	16.93	14.78
L Leu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg .	3	3.64	2.61
S Ser	7	4.74	6.09
T _. Thr	9	7.07	7.83
∨ ∨al	14	10.78	12.17
W Trp	0	0.00	0.00
Y Tyr	. 2	2.54	1.74
B Asx	0.	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.11 b

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poly (A) tail

FIG. 4.12 a

Composition of 7-5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 mlcrogram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4,22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

M/holo	Protein	Composition	Analyeie
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Whole r totelit compositi	Number	% by	% by
Amino Acid(s)	count	weight	frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
l lle	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gin	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	. 2	2.54	1,74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.12 b

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FIG. 4.13

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FIG. 4.14

	(kDa)										(% mole)	mo	le)						•			
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Tm	12.86	117	3.2	3.0	3.4	4.4	8.5	2.0	4.4	28.9	3.1	3.9	3.8	3.2	N N	10.7	15.0	3.9 3.8 3.2 ND 10.7 15.0 3.6 14.9 6.8	14.9	8.9	6.3	57.3
12.80 Tm	13.17	116	3.13	2.21	4.47	5.16	10.5	1.0	4.3	29.11	1.73	3.24 0		0 1.4 0	1.4	0	0	7.12 15.6 3.31	15.6	3.31	6.14	32.14
13.17	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	1.78 3.32 2.54 2.14 0	2.14	0	0	0	2.43 18.0 4.75 7.08 32.23	18.0	4.75	7.08	32.23
2-3	12.84 115	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	4.41 27.26	1.78	3.32	1.78 3.32 2.54 2.14 0 0	2.14	0		0	2.43 18.0 4.75	18.0	4.75	7.08 32.23	32.23
3-4	12.84 115	115	2.41	3.02	3.44	5.29	11.6	1.02	4.41	28.04	1.78	3.32 2	2.54 2.14 0 0	2.14	0		0	2.43	18.0 4	4.75	7.09 32.24	32.24
3-9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54 2.13 0 0	2.13	0	0	0	3.64	16.9	4.74	7.07 32.38	32.38
7-5	12.84 115 3.21	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	1.78 3.32	2.54 2.14 0 0	2.14	. 0		0	2.43 18.0	18.0	4.75	7.08 32.23	32.23

FIG. 4.15

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Tm 2-2 3-4 3-4 3-9 Tm Tm B1 AFP.
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3-9
7-5
Tm
B1
B2
```

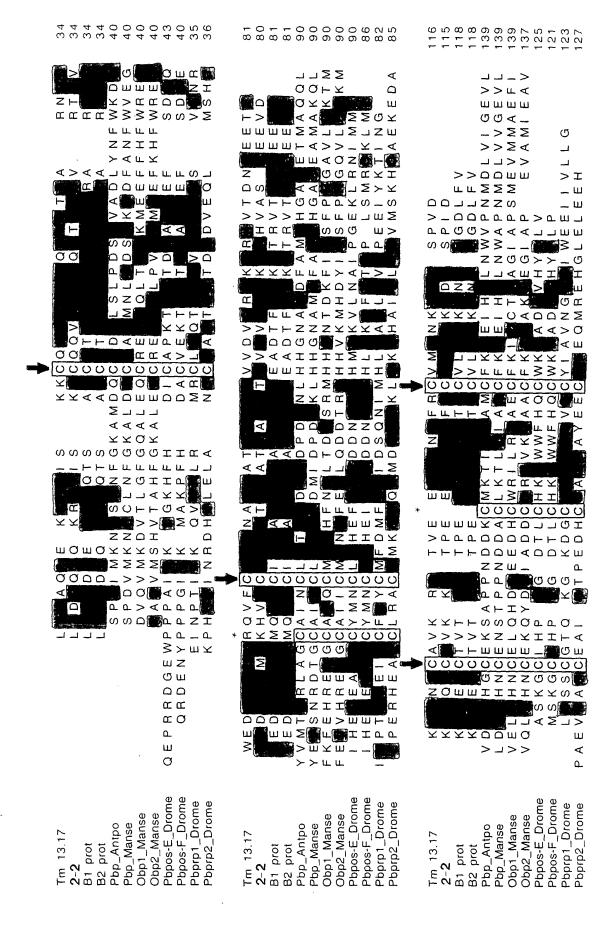
XXXXXX

>> LL ___>00 $\overline{\Omega}$ Ω Ω Ω Ω Ω Ω Ω Ω Π Π DDDDDYZZ a a a a a a a o **ΧΧΧΧΧΧΧΧ**α OOOOOZDDZ 0000**0**X**XX**0 **ナイト 2 人人人人人** ___>>> 00>000000 XXXXXXXXXX $\vdash\vdash\vdash\vdash\vdash\vdash\gt>$ 00000Z**wwo** ~ ~ ~ ~ ~ L L L C AAAA>AAO FFFFFFF шшшшшаат ய ய ய ய ய ய **ய ய** 🗷 0000000000 HIERERIE 44440000

13.17

2-2 2-3 3-4 3-9 7-5 Tm **B1 B2** FIG. 4.16

< :

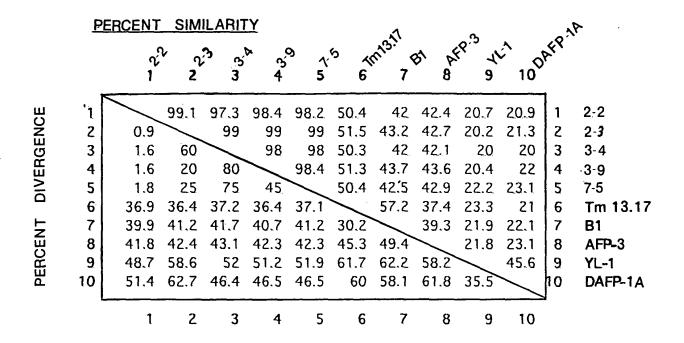


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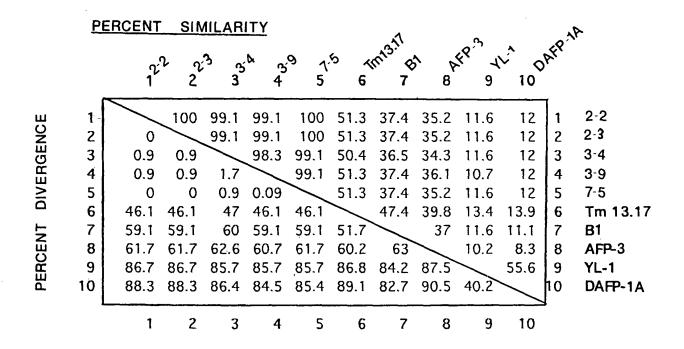
FIG. 4.18

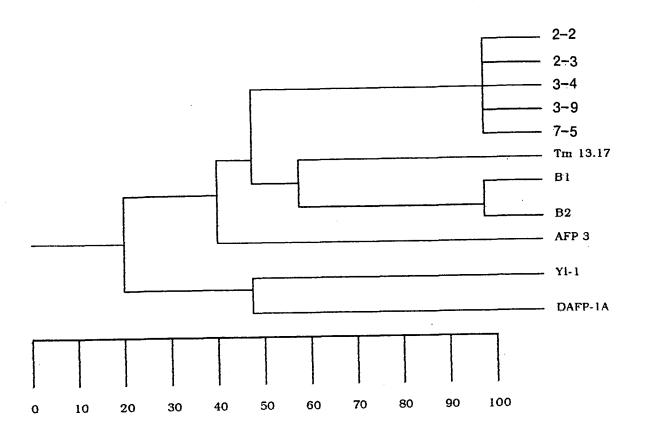
NUCLEOT E SEQUENCES





AMINO ACID SEQUENCES





% Nucleic Acid Identity

FIG 4.20

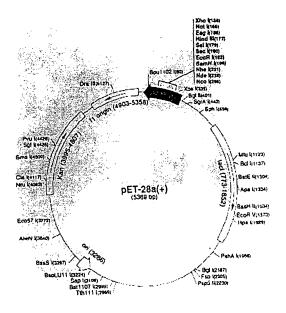
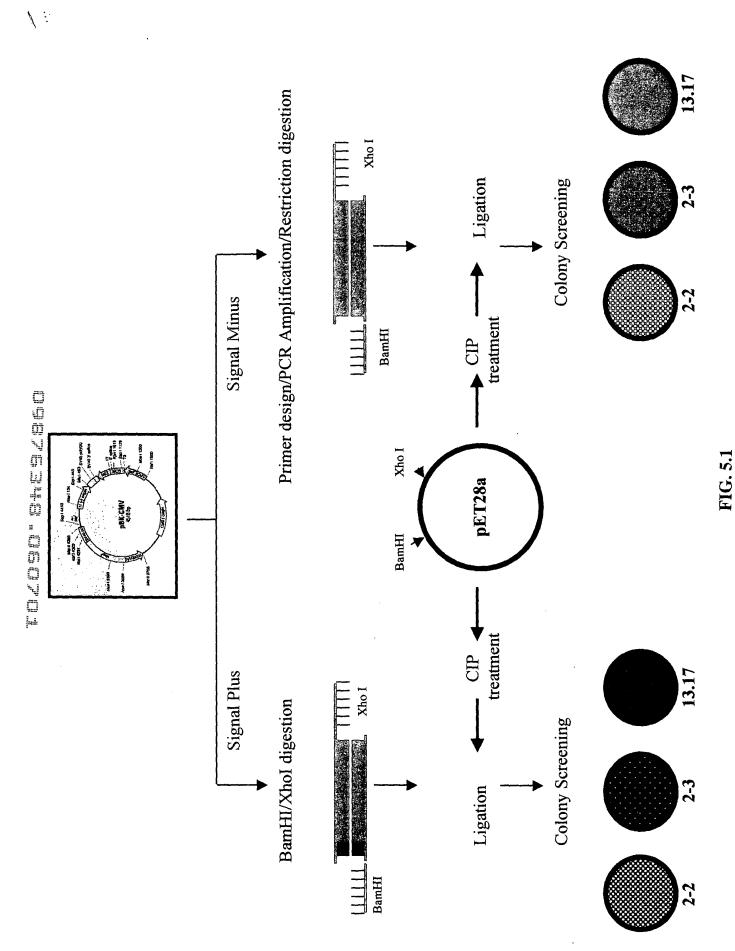


FIG. 5.0



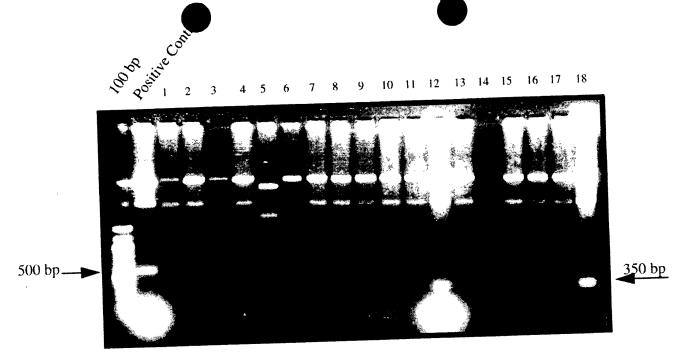


FIG. 5.2

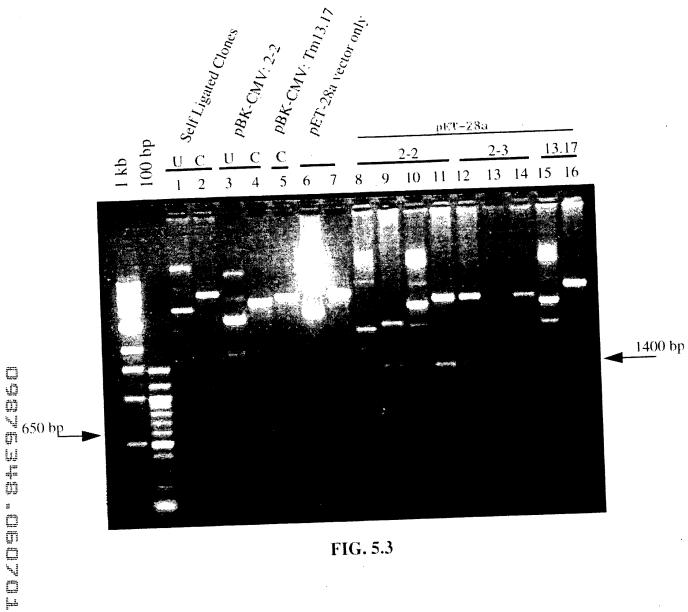


FIG. 5.3

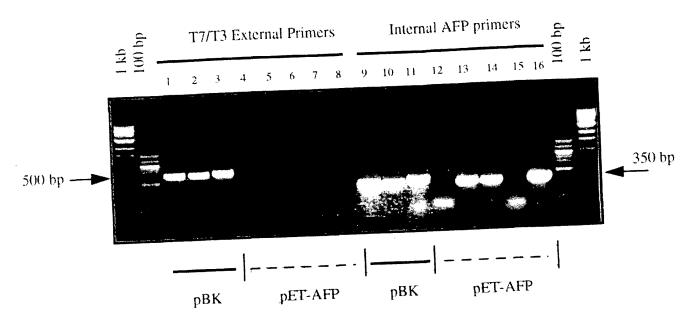


FIG. 5.4

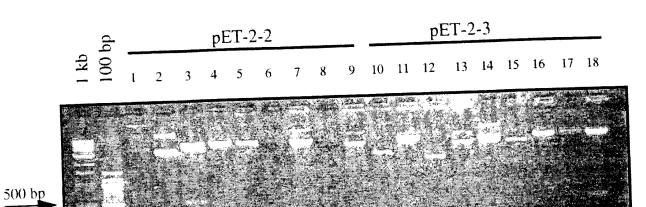


FIG. 5.5

FIG. 5.6

٠....

His-tagged Clone 2.2 with signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -55 -50	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -45 -35	141
AFP Star GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG Gly Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met -30 -25 -20	t Codon 186
AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT Lys Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala -15 -10 -5	231
N-terminal of mature AFP CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser 1 5 10	276
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp 15 20 25	321
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys 30 35 40	366
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala 45 50 55	411
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val 60 65 70	456
GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val 75	501
AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys 90 95 100	546
Stop Codon ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp * 105 110 115	595
Polyadenylation signal Poly-A tail TTTGACTGAA TTTTGAC <u>AAT AAA</u> GGTAATA TCGTTATGTA AAAAAAAAA	645
AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT	681

15

His-tagged clone 2.2 without signal sequence

TTGT	TAGO	CGG A	TGG	ATTC	CC CT	CGTA	AGGGG	ATA	LATTI	TGT	TTAC	TTTF	AAG		50
AAGO	AGAT	PAT A	ACC A		GČ I	AGC A	AGC C Ser H	AT C				lis F			96
		CTG Leu													141
		CAA Gln						CTC	ACC	GAC	GAA		ATA	CAG	186
		AAC Asn 10													231
TCC Ser	CAA Gln	GAG Glu 25	ACG Thr	ATC Ile	GAC Asp	AAA Lys	GTC Val 30	CGC Arg	ACA Thr	GGT Gly	GTC Val	TTG Leu 35	GTC Val	GAT Asp	276
		AAA Lys 40													321
		GCA Ala 55													366
		CTG Leu 70													411
		AAG Lys 85													456
		ACC Thr 100													501
	Ile	GAT Asp	TAA	OCTC		ACC 2	ACCAG	CCAC	CA CO	CACTO	GAGA'	r			543

His-tagged clone 2.3 with signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -55 -50	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -45 -35	141
AFP Start GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met -30 -25 -20	Codon 186
AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT Lys Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala -15 -5	231
N-terminal of Mature AFP CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser 1 5 10	276
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp 15 20 25	321
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys 30 35 40	366
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala 45 50 55	411
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val 60 65 70	456
GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val 75	501
AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys 90 95 100	546
Stop Codon ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp * 105 110 115	595
Polyadenylation signal Poly-A tail TTTGACTGAA TTTTGAC <u>AAT AAA</u> GGTACTA TCGTTATGAA AAAAAAAAA	645
AAAAAAACTC GAGCACCACC ACCACCACCA CTGAGAT	682

1.

His-tagged Clone 2.3 without signal sequence

TTGI	TAGC	GG A	TGGA	ATTC	C CI	CGTA	(GGGG	ATA	ATTI	TGT	TTAC	TTTT	AAG		50
AAGG	AGAT	'AT A	CC A	ATG G	GČ A	AGC A	Cod GC C Ger H	AT C	CAT C	CAT C	CAT (lis F	lis F	CAC A His S	AGC Ser	96
							AGC Ser								141
							TCC Ser	CTC		GAC	GAA	CAG	ATA	CAG	186
							GAA Glu 15								231
							GTC Val 30								276
							GTC Val 45								321
							GAC Asp 60								366
							AGC Ser 75								411
							AAG Lys 90								456
							TAC Tyr 105								501
		GAT Asp 115	TAA	CTC		ACC 1	ACCAG	CCAC	CA CO	CACTO	GAGA'	r			543

FIG. 5.10

His-tagged Tm 13.17 with signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -65 -60 -55	96
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -50 -45 -45	141
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile -35 -30 -25	186
AFP Start Codon CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser -20 -15 -10	231
N-terminal of mature AFP CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT Leu Ile Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile -5 5	276
GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly 10 15 20	321
GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu 25 30 35	366
GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn 40 45 50	411
GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu 55 60 65	456
AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu 70 75 80	501
AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu 85 90 95	546
ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys 100 105 110	595
Stop Codon TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG Phe Ser Pro Val Asp * 115	643
Polyadenylation signal Poly-A tail TGTGCTTTAC ATATAAA <u>AAT AAA</u> GTGTTTC TGATGTAAAA AAAAAAAAA	693
AAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT	743
TCCACCCTC GAGCACCACC ACCACCACCA CTGAGAT	777

His-tagged Tm 13.17 without signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG										
His-tag Start Codon AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC Met Gly Ser Ser His His His His His Ser -30 -25	96									
AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly -20 -15 -10	141									
N-terminal of mature AFP GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys -5 1 5	186									
CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser 10 15 20	231									
CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp 25 30 35	276									
CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly 40 45 50	321									
CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu 55 60 65	366									
AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile 70 75 80	411									
ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val 85 90 95	456									
TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser 100 105 110	501									
Stop Codon CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT Pro Val Asp * 115	543									

FIG. 5.12

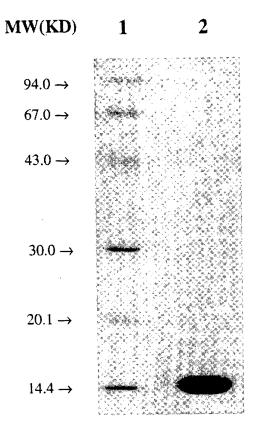


FIG. 6.0

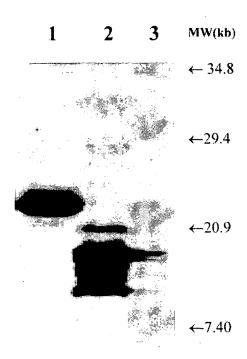


FIG. 6.1

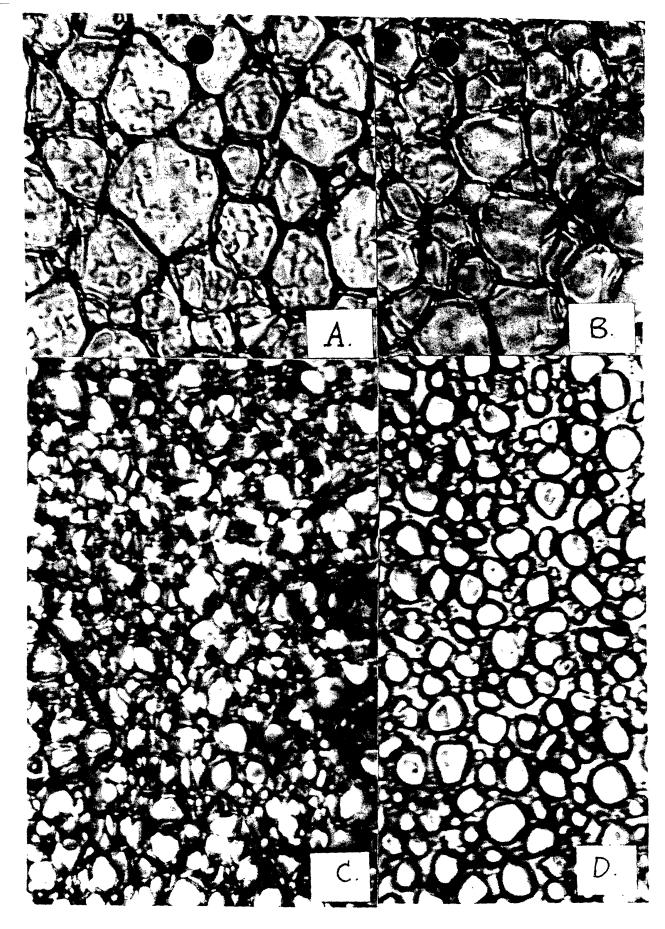
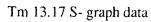
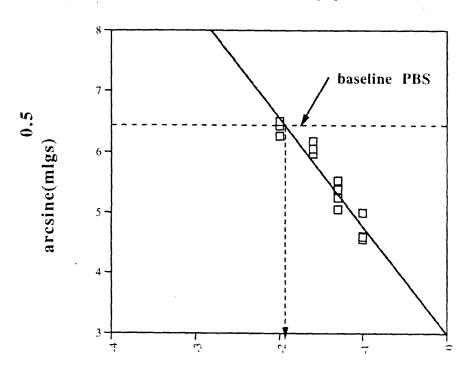


FIG. 6.2





log dilution

FIG. 6.3

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
Α	Alanine	Ala	aliphatic	mod. hydrophobic	low
В	Asp or Asn	Asx			
С	Cysteine	Cys	sulfhydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E F	Glutamic Acid	Glu	acidic	highly hydrophilic	high
F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
H	Histidine	His	basic, imidazole	highly hydrophilic	high
1	Isoleucine	lle	aliphatic	hydrophobic	low
J					
K	Lysine	Lys	basic	highly hydophilic	high
L	Leucine	Leu	aliphatic	hydrophobic	low
М	Methionine	Met	sulfhydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
0					
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q	Glutamine	Gin	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
T	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
U					
V	Valine	Val	aliphatic	hydrophobic	low
W	Tryptophan	Trp	aromatic	highly hydrophobic	low
X					
Υ	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
Z	Glu or Gln	Glx			
		ACD	Any Acidic		
		ALP	Any Aliphatic		
		ALH	Any Aliphatic Hydroxyl		
		ARO	Any Aromatic		
		BAS	Any Basic		
		HY-	Hydrophobic		
		HY+	Hydrophilic		
					•

FIG. 7.1

Position	Tm 12.84-2.2	Tm 12.84-2.3	Tm 12.84-3.4	Tm 12.84-3.9	Tm 12,84-7.5	Concensus of Tm 12.84	Tm13.17	Concensus with Tm 13.17	81	Concensus with B1	AFP-3	Concensus with AFP-3
1	A	Ā	Č.	· <u>A</u>	Ā	A	A	A		A	С	N
2 3	C G	C G	Ġ	G G	C G	C G	G A	N R		N R	A G	N A
4	A G	A	A	Å	A	A	С	N		N	A	N
5 6	ç	G C	G	G C	G	G C	T A	N		N N	T C	N N
7 8	^	Â	Â	Â	A	A A	C T	N N		N N	C G	N
9	Ä	A	A	A	A	A	Ä	A		A	Á	Ä
10 11	Ā	A	Â	A	A	Å	A G	A		A R	A G	A R
12	:	•		:	•	•	•	•		•	•	•
13 14	A T	A T	A T	A T	A T	A T	A T	A T	A? T?	A T	A T	A T
15 16	G A	G A	G A	G A	G	G	G	G	G?	G	G	G
17	Ä	A	A	A	Â	Â	A	A		A A	A	A A
18 19	A C	Å	A C	A C	A C	Å C	G T	R Y		R Y	G C	R Y
20	T	Ť	Ŧ	7	т	T	Т	Ť		Т	T	T
21 22	C	C	C	C	C	C C	G C	C/G C		C/G C	c c	C/G C
23 24	T G	r c	Ç.	T C	r c	T	Ť C	T C		Ť C	T	Т
25	т	T	T	T	т	C T	T	T		T	c	C Y
26 27	T G	T G	T G	Ť G	Ť G	T G	G T	N N		N N	T C	N
28 29	T G	Ť G	T G	T	T	T	T	T		T	Ť	T
30	C	Ç	C	G	G C	G C	G T	G Y		G Y	G T	G Y
31 32	T T	T T	T T	Ť	Ť	T T	C T	Y T	C T	Y T	C T	Y T
33 34	7	T	τ	T	Ť	T	A	T/A	T	T/A	Ť	T/A
35	G	G C	G C	G C	G C	G C	A T	R Y	A. C	A Y	G T	R Y
38 37	G T	T T	Ť	T T	G T	N	C	N	τ	N	Ċ	N
38	T	т	T	т	7	Ţ	T C	Ť Ý	T C	Ť	C T	Y
39 40	C G	G G	G G	c G	G G	C G	C	C G/C	r C	∂\c A	e C	G/C
41	c	С	С	C	С	C	T	Y	т	Y	Ţ	Υ
42 43	C G	C G	C G	C G	G G	G	G A	C A	C A	C R	Ť G	Y FI
44 45	C C	C	C C	C C	c	C	T	Ÿ	Ţ	Y	C	Ÿ
46	A	A	A	A	A	A	r c	N	С	Y N	C T	Y N
47 48	T C	T C	Ť C	T C	Ť G	T C	T G	T G/G	T G	T C/G	Ť G	T C/G
49 50	G	G.	G	G	G	G.	T·	N	т	N	G	N
51	T C	T C	r C	C C	t C	Ť G	T G	T C/G	T G	T C/G	T C	T C/G
52 53	A T	A T	A T	A T	A T	A T	G T	A	G	R	Ġ	R
54	C	С	C	Ċ	Ċ	Ċ	С	r c	T G	r c	c c	Y C
55 58	G G	G.	G G	G G	G G	G G	Å C	A GVC	C C	N G/C	G C	N G/C
57 58	A G	A G	A G	A G	A G	Ā G	A G	A	A	A	G	R
59	C	С	С	С	c	c G	G T	G Y	G. T	G Y	A C	A Y
60 61	T C	T C	T G	T C	T C	C	T C	r C	C	T C	C T	Y
62 63	A G	A G	A G	A	A	A	A	A	A	A	A	. Å
64	G	G	G	G. G	G G	G G	G G	G G	G	G G	G G	N G
65 66	C T	C T	G T	. C T	C T	C T	C C	C Y	c	C Y	c	C
67 68	Ç	C T	C	C	Ċ	Ċ	C	C	A	N	·	Ň
69	С	Ċ	r C	t C	T C	T C	T G	T C/G	T A	T N		T N
70 71	Å	Ĉ	Å	A C	A C	A C	A C	A C	Ĉ	A C		A
72 73	С	С	С	Ç	C	С	С	С	T	Y		C Y
74	G A	G. A	G A	G.	G.	G A	G A	G A	C A	G/C A	G A	G/C A
75 76	G G	C G	C	T G	C G	y G	G G	N G	G	N	A	N
77 78	A	A	A	A	A	A	С	N	G A	N G	Å C	R N
79	Ĉ	Ĉ	Å C	A C	A C	Å C	Č	Ĉ	A G	A C/G	A C	A C/G
80 81	A G	Å	A G	A G	A G	A G	A	A B	A	A	С	Ν '
82 83	Α	A	A .	A	A	A	Ä	A	C	N N	T C	N N
84	T A	T A	T A	T A	T A	T A	Ť	₹ A/T	T T	T A/T	G T	N A/T
85 86	C.	Ç	Ç.	C	Ç	ç	G	C/G	G	C/G	G	C/G
87 88	å	A G	ä	Å	A G	A G A A A	A G	Å G	A G	A G	A G	A G
89	A A A G	A A A G	â	A	Â	Â	Â	A A N	C T A C T	N TA	A	N
90 91	^	^	•	Å	A	A	Ä	Ä	À	Ä	G	A/T R
92	Ģ.	â	â	G	A G	G	A C T	N	C T	N	C T	N N
93 94	G. A	G.	G. A	G A	G A	G	G	G.	G	G	G	G
96	A	Ā	Ā	A	Â C	Â	A	Â	č	N N C	A A	N N
93 94 96 96 97	G A A G A T C	A A C A A G A T C A G C A A A G A A T	A G A A A A G G A A C A A G A 7 C A G C A A A G	G A A	C A	C A	С	N G A A C A A G A T C A G C A A A R A R A R A R T	G C C C C A A A C C A G C G C A G	C N	G C	Y N
98 99	Å	Å	A	A G	A	A G	A A G	Ä	Ă	A	A	Ä
100	Ā	Ã	Ž	Â	G A T	Ą	Å,	A A	Ä	A A	G C	A R N
101 102	ć	C	T C	Č	r C	A T C	c T	ŗ	C	Å Y	Å	N
103 104	Å	Å	Á	Å	Ã	Ă	Ă	Ă	Ă	C A	A	Å
105	A G C A A G	č	č	С	g C	A G C	A , C A G C	G.	G C	G	G C	N C A G C R N
106 107	A	Å	A	Â	A	A	Â	Á	Ğ	C R	G	R
108 109	â	Á	À	Ã	Å Å G	A A G A R		Â	Ä	N A R	Å C	N
110	Α .	Ā	Ā	A	G A	G A	Â	R A	G ▲	R A	G	R
111 112	A T	A T	Â	Ā	A G T	Ŗ	Â	R	A G T	R	С	N N N T
113	G	Ġ	Ġ	G	G	T G	G	Ğ	T G	T G	Ť G	T O
114 115	C C A G	G C A G	G C C A G	C C	c	C C	C C	G Y C A R	G G	Y	С	G Y
116 117	A G	A	Å	A	Å	Å	Â	Ă	A G	N A	A	N A Fi
	4	3	u u	G	G	G	A	R	G	A	G	A

FIG. 7.2

				_					_			
Position 118	Tm 12.84-2.2 C	Tm 12,84-2,3 C	Tm 12.84-3.4 C	Tm 12.84-3.9 C	Tm 12.84-7.5 C	Concensus of C	Tm13,17 A	Concensus with N	B1 A	Concensus with N	AFP-3 G	Concensus with N
119	A	A	A	Α	A	A	A	A .	С	N	C	N
120 121	G G	G. G	G G	G G	G B	G G	T G	N G	T G	N G	T G	N G
122	T	T	Ť	. A	Т	T/A	A .	T/A	A	T/A	Α	A/ T
123 124	G. T	G T	G T	G T	G T	G T	A	R T/A	A	A T/A	. T	R T/A
125	С	С	С	С	С	C	Ĝ	C/G	G	C/G	С	C/G
126 127	C G	C G	C G	C 6	C G	C G	T G	Y G	T G	Y G	T G	Y G
128	Ğ	Ğ	G.	Ğ	Ğ	G	G	G	Ğ	G	Ġ	G
129 130	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A	A G
131	T	T	Т	T	T	T	ī	T	T	T	G T	Ť
132 133	G T	G T	G T	G T	G.	Q 7	G T	G	G T	G T	- A	R
134	Ċ	ć	C	С	Č	ć	ć	T C	ć	T C	T C	Č
135 136	c c	C C	C	C	c c	C	G C	C/G	C G	C/G C/G	T	N C/D
137	Ă	Ă	Ă	A	Ä	Ä	Ä	Ä	Ā	A	G A	C/G A
138 139	A G	A G	Å	A G	A G	A G	A G	A G	A G	A G	A G	A G
140	A	A	A	A	A	A"	A	A	A	A	A	A
141 142	G. A	G A	G A	G. A	G A	G A	G A	G A	C G	G/C R	G T	G/C N
143	С	С	С	C	C '	C	T	Y	T	Y	C	Υ
144 145	G A	G A	G. A	Q. A	G A	G A	C A	G/C .	C A	G/C A	C	G/C N
146	7	T	T	Ť	Ť	T	Ţ	T	T	7	T	7
147 148	C G	C G	C G	G	C G	C G	Â	N B	A A	N R	C	N R
149	A	A	٨	A	A .	A	C	N	A	N	A	N
150 151	C A	C A	C A	C A	C A	C A	C A	G A	G A	C/G A	C A	C/G A
152 153	<u>^</u>	٨	Ą	A	A	A	A	A	G	R	A	R
154	Å G	A G	å	A G	Å	A G	· A G	A . G	A G	A G	G G	R G
155	т	Ţ	T	Ť	T	Т	C	Y	С	Y	т	Y
156 157	c c	c	c c	C C	C C	C C	t C	č	T C	C Y	c C	Ç Ç
158	G	G C	G	G	G	G	Ġ	G	Ğ	G,	G	G
159 160	C A	Á	C A	C A	G A	C A	C A	C A	C A	C A	C A	C A
161 162	C A	C A	C A	Č A	A C A	C A	A C	N N	A	N N	A C	N N
163	G	G	G	G	G	G	G	G	G	G	С	G/C
164 165	G T	G T	G T	G T	G T	G. T	G T	G T	G T	G T	G T	G T
168	G	G	G	G	G	G	Ġ	Ğ	G	G	G	G
167 168	T G	T C	Č	T C	T C	T C	G A	T/A C	Ĉ	T/A C	A	T/A N
169 170	Ť Ť	T	Т	Ŧ	Ţ	T	T	T	Ţ	7	G	N
171	G	đ	T G	T G	T G	T G	G G	N G	T G	N G	A	N R
172 173	G T	G T	G T	G T	G T	G T	G	G	G	G	G	G
174	С	C	C	Ċ	С	C	A G	T/A C/G	A G	T/A C/G	T G	T/A C/G
175 176	G A	G A	G A	G	G. A	G A	G A	G A	G A	G A	G A	G A
177	T	С	C	С	C.	Y	С	Y	С	Y	С	Y
178 179	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A	G A
180 181	T C	T	7	T	Т	T	Т	٢	С	Y	C	Y
182	С	C	C	C	C	C	C	c c	C	C C	C	C
183 184	C A	C A	C A	С	С	Ç	r	Y	С	Y	С	Y
185	A	Ä	Â	Â	A	A A	Â	Â	Â	A	A	A
186 187	Â	Â	Â	A	Â	A A	A C	A N	A C	A N	A C	A N
188	Т	Т	T	T	τ	T	T	Ť	T	т	T	T
189 190	G A	G A	G.	G A	G A	G A	G A	G A	G A	G A	G A	G A
191 192	A G	A G	Å	A	A	A	A	Ä	A	A	A	A
193	A	A	Å	G A	G A	G A	A C	A N	A	R N	A G	R N
194 195	A G	A G	A G	A G	Å	A G	G C	R G/C	Ţ	N	Α	N
196	č	č	č	č	č	Č	č	C	G	G/C C	G C	C G/C
197 198	Ĉ	Ĉ	Å	A C	ĉ	A C	A	N .	Ą	A N	A T	A
199	G	G	ğ	G	G	G	Ĝ	G	ć	· G/C	Ġ.	g/c N
200 201	C C	C C	č	T C	C C	T C	7	Ţ	Ť	T Y	C T	Y
202 203	C	ç	ç	С	C	ğ	Ì	Ý	ċ	Ý	Ť	Ý
204	C C	Ċ	ç	C C	C C	Č	7	Ť Y	Ţ	T Y	T C	T v
205 206	C T G	T G	Ţ	C T	C T	T	T	T	T G	Y T G	C T G	Ť
207	C T	Ç T	G C T C T	c	Ċ	G	G C	G C	G	G C	G C	G C
208 209	T T	Ť	Ţ	Ţ	Ţ	6 1 7 6 1 6	G	N	C A T T	C N T	Ā	Ň
210	Ċ	Ċ	ċ	ċ	ċ	ċ	T G	T C/G	Ť	N	ć	T N
211 212	Ţ	Ť	Ţ	Ţ	Ţ	Ţ	G	C/G N C	Ţ	N	Ţ	N
213	C G A	Ğ	C G	Ğ	Ğ	Ğ	C	G/C	T C	G/C	Ğ	G/C
214 215	<u>^</u>	Â	^	Å	•	A	A G	A	A	<u>ر ۸</u> ,	À	/ As
216	ä	Ĝ	Ĝ	Ĝ	Ĝ	Ĝ	G	A R G	Ĝ	(Å' .',	Ĝ	G G
217 218	Â	A	A	A G	A ·	A R	Å Å Ç	A P	G	FI N	A	R
219 220	A .	A	Å	Ã	À	Ä	ć	· 🙀	Ă	N	Ā	N
221 222	A G A A C T	C G A A G A A A C T	A G A A C T	Ĉ	G C T T C T C G A A G A A A C T	Ĉ	G C	. N R C Y	C T	N Y G/C (A, C) , , , , , , , , , , , , , , , , , ,	AT CT T G A A G A G A G C C G	N Y
222 223	T G	T a	T G	T	Ţ	Ţ	Ċ	Ý	Ċ	ý	ć	Ý
224	G G	Ġ Ġ	G G	Ğ	G	G	G G	G	G A	Y G R	G G	G R
225 226	A G T	A G	A G T	A G	A G	A G	1	A/T G#	A A G G C A C T C G A A A T	A/T	G A T	A/T
227 228	Ť	Ŧ	Ť	G C T T C T C G A A G A G A A C T G G A G T G	G A G T G	ARAACT GGAGT G	C T	G G A/T G/C T	Ť	A/T N T	Ť	T.
228 229 230	G G	G G	G.	G G	G G	G G	G G	G G	A G	R G	C	Y T G C Z T Z Z Y G G A G G R X Z Y Y G R Z Z T X R Y
230 231	C A	C A	G G A C C	G C A C C G	G C A C C G	G . A A C	С	C	Ť	-γ	A T	Ÿ
231 232 233	A	Ã	Â	Â	Â	Â	C A	, C	T G G G	N R	. C	R N
233 234	C C	A C C	C	c c	c	c	C B	. c	Č.	R C	A C	N
234 235	Ğ	Ğ	ă	Ğ	Ğ	Ġ	G	C/G G	G	Ç/G G	C G	C/G G

FIG. 7.2 Cont.

Position	Tm 12,84-2.2	Tm 12.84-2.3	Tm 12.84-3.4	Tm 12.84-3.9	Tm 12.84-7.5	Concensus of	Tm13.17	Concensus with	81	Concensus with	AFP-3	Concensus with
238 237	Å	A	Å	A	A A	A	A	A	A A	A A	C	N N
238	G	G	G	G	G	Å G	A T	N	т	. N	A	N
239 240	C C	C	C	C	C	C	G	C	C G	C C/G	G T	C/G N
241	G.	G	G.	G G	G	G.	G	G	G	G	G	G
242 243	G A	G A	G A	A	G A	G A	G A	G A	G A	G A	G T	G A/T
244 245	G	G. A	G	G	G	G	G	G A	G	G	G A	G A
246	Ĉ	С	Å C	Å C	Ĉ	Ĉ	A G	C/G	Â	A N	A	N
247 248	A C	Å	Å	Å	Å C	A C	G T	R	A T	R Y	T T	N Y
249	C	С	С	C	C	C	G	C/G	Ť	N	c	N
250 251	A	Â	A	Å	A	A	G T	R A/T	G A	R A/T	C A	N A/T
252	T	T	7	Т	т	Ŧ	G	N	G	N	A	N
253 254	G T	G T	G T	G T	G T	G T	G T	G T	G C	G Y	C T	G/C Y
255 256	G G	G G	G G	G G	G	G	C G	G/C	С	g/c	G	G/C
257	A	Ā	A	A	G A	G A	A .	G A	G. A	G A	G A	G A
258 259	G G	G G	G G	g G	G G	G G	C G	G/C G	G A	G/C R	c	G/C N
260	T	τ	Т	T	Т	7	T	Ť	C	Y	A	N
261 262	.C	Å C	Å C	A C	A C	A C	G T	R Y	G T	R Y	G A	N N
263	. 7	τ	T C	T	T	7	τ	7	7	T	T	7
264 265	C A	C A	A .	, C	C A	C A	G A	C/G A	C A	C/G A	T A	N A
266 267	A A	A	Ä	A A	A A	A A	G G	R R	A G	R R	A G	R
268	a	G	G	a	a	G	G	G	G	G	A	R
269 270	C	C G	c c	C	C	C	A G	N C/G	A G	N C/G	C G	N C/G
271	A	A	A	A	A	A	A	A	A	A	A	A
272 273	Ġ	Ğ	A G	A G	A G	A G	A G	A G	A G	A G	A	A R
274 275	C T	Ç	С	С	C	С	G	C/G	т	N	т	N
276	G	T G	T G	Ğ.	Ť G	Ť G	T G	T G	T G	G G	Ť C	G/C
277 278	Å	Á	Â	Á	A	A	. A	A R	Å	Â	A	A N
279	G	G	G.	G	G	G	G	G	G	G	G	G
280 281	C A	C A	C A	C A	C A	C A	A A	N A	A G	N R	G. A	N R
282	Т	τ	A T	A T	T	Т	G	N	G	N	A	N
283 284	G T	G T	G T	G T	G T	G T	G T	G. T	T G	N N	A	N N
285 286	G G	G G	G G	G G	G G	G G	C A	G/C R	A C	N	C	N
287	С	C	C	C	C	C	ĉ	С	Ä	N N	T C	N N
288 289	G A	C A	C A	C A	C A	Ç A	T G	Y R	A	N R	Ť G	N R
290	G	G	G	G	G	G	A	В	c	N	A	N
291 292	С	С	С	С	С	С	C A	C A	G A	C/G A	G	C/G R
293							A	A	T	A/T	Α	A/T
294 295	G	G	G	G.	a	G	G G	C G	G A	C/G R	Ţ	N N
296 297	A C	A C	A C	A C	Å C	A C	A C	A C	T G	A/T C/G	С	N
298	G	G	G	G	G	G	G.	G	A	R	G G	C/G R
299 300	Å	A	A .	A	A	A A	A	A	A G	A B	A G	A R
301 302	G A	G A	G.	G.	G	G	G	G	A	R	A	A
303	G	A	G	Â	A G	A	Å	A R	Ā	A R	A G	A R
304 305	G T	G T	G T	G T	G T	G T	Å	R Y	G C	R Y	G T	R Y
306 307	G G	G G	G	G	G	G	т	N	G	N	С	N
308	A	A	G A	G A	G A	G A	G A	G A	A G	R R	G A	A A
. 309 310	C A	C A	C A	A C A	C A	G A	G A	C/G A	A	. N	Ť	N
311	A	A	A .	A	A	A	A	A	A G	A R	G A	R R
312 313	G A	G A	G A	G. A	G.	G. A	A	A A	A T	R A/T	C T	N A/T
314 315	Ť C	T	Ť	7	7	7	т	T	т	T	Ť	т
316	G	G G	C G	C G	G G	C G	C A	C R	G T	C/G N	G	C/G N
317 318	T G	T G	G.	T G	T G	T G	T C	T G/C	C G	G/C Y	T T	Y
319 320	C A	C	C	С	С	C	A	N	Ä	N	G	N N
321	Ĝ	A G	A G	A G	A G	A G	A T	Å	G A	R N	C	N N
322 323	^	Å	A	^	A	Ą	A	Ą	A		Ā	A R
324	A G T	G	G	A G T G	A G T	A G T	Å G	A G	G T	R N N	A	R N
325 326	G.	T G	G G	T G	Ţ G	T G	T G	T G	G C	G/C	A T G	N
327 325	c	С	G G T	c G	С	С	С	С	Ă	N	T	G/C N
329	Ť	G 7	7	7	G. T	G T	G	G. Y	A C G	G/C N	G C G T C	G/C N
330 331	G G	G	G G	G G	G G	G	C G	GUC G T C	Q T	G/C	ç	G/C N
332	Ť	G T	Ţ	T	Ţ	G. T	T C	T T	Ġ	N N	G T	N N
333 334	G C G T G G T C A A	. A . A	· A	C A	C A	C A	C	Ç.	A	N	¢	N
335 336	Ä	, <u>v</u>	Ä	Ä	Ã G	A	A A G	A . A G	A C T	N N	A G	N N
336 337 338	G A		G A	A G A G	G A	G A	G A	G.	G A A C	G	G	G A
339	A	A	Å	A	A G	A .	A G	A R	Ä	A R	A A G	R
340 341	G	Ğ	G	G	G	G G	A G	R G		N R	G	N
341 342	c c	C C	C	G C A C	a C	С	Ä	N Y	ç	N N	Ā	R N
343	Ă	Ă	Ă	Ă	C A C	C A C		Å	Ĉ	N N	G A	N N
342 343 344 345 346 347	A A G G C C A C A C C	A G A G G	C A A G A A G G C C A C A C C	C A	C A	C A	A C T	C A/T	Ţ	N Y N	G A C A C	N Y
346	ç	Ċ	Ċ	A C C	Å	Å C	G	ã	č	C/G	ċ	N C/G
348	Á	A G	A G	G A	C A	C A	Ť	A/T C/G Y A/T G	G. G	N N A	c T C	N
348 349 350	G ▲	G A	G	A G	A G A	A G	G	G	A C A C T C C G G A A G	Ä	ċ	N
351 352	A G	ā G	A G G	A G G	G	A G	Â	A	å	A R R	A G C	A
352 353	G A	G.	G A	G A	G. A	G A	G A	G A	A T	A/T	Ç	N
						•••		-		~ 1	A	A/T

FIG. 7.2 Cont.

Position 354 3554 3554 3555 3567 3575 358 358 359 359 350 359 351 358 358 359 357 378 378 378 379 330 331 334 335 336 336 336 337 379 330 330 340 401 402 403 404 405 406 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 407 408 409 410 411 412 424 425 427 428 429 420 421 421 422 423 424 425 426 427 428 429 420 421 421 422 423 424 425 426 427 428 429 420 421 421 422 423 423 424 424 425 427 428 429 420 421 421 422 423 423 424 425 426 427 428 429 420 421 421 422 423 423 424 425 426 427 428 429 420 421 421 422 423 423 424 425 427 428 429 429 420 421 421 422 423 424 425 427 428 429 429 420 421 421 422 423 424 425 427 428 429 429 420 421 421 422 423 424 425 427 428 429 429 420 421 421 422 423 424 444 445 445 446 447 448 448 449 449 449 449 440 441 441 441 442 443 444 445 445 446 447 448 448 449 449 449 449 449 449 440 441 441 441 442 443 444 445 446 447 448 448 449 449 449 449 449 449 440 441 441 441 441 445 446 447 448 448 449 449 449 449 449 449 449 449	2.84-2.2 Tm	TO TO THE TOTAL GARAGE GENERAL ACCUTATE AND CONTRACT TO THE CONTRACT OF THE CO	TM	39 12.4 ACCGGCTTATGACACCTTCAAGTGTATTTACGACAGTAAACCT GATTTCTCTCCTGCTATTGAT. TAAATTGTTTTTGACTGAATTTTGAC	TM	Concentsus of A A A A A A A A A A A A A A A A A A	TM13.17 ACGGGTGTTCAAATACTTTCAAAATGAAAAAAAAAAAAA	Concernsus with ACGGGYNTATYRAYACYTTCAAARNNTRTYAACGRANARYAAARCCTARTTCTCTCTCTCTCTACTACTAYTCTTAGNNNRATRNTTYRANTGGTGTGCTTACCTACTACTACTACTACTACTACTACTACTACTACT	BACGCCATTTGAAGTTACCAAATGTGTATTGAAGGACAAGCCCA ATTTCTTTGCAGACTATT TGTCTGAAAGCTTTTGTGCACAA	CONCERNIC CONCER	AFCT C C T C A G C C G A C T T C T T T A A A T G C G A T A A C A G G T C G	Concensus with NNG GIRN NNNN NA ANNN NNNN NNNN NNNN NNNN NNN
441 442 443 444 445 446 447 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 464 465 466 470 471	T G	TT G A C	TTTGACC	TTTGACC	T T G A C	T T G A C	T C A A T G	T T Y R A N T G G T G C T T T A C A T A A A A		A N T G G	TT AT A AT GT A A A C C T T G C C A A T T T T T T G A A C	TIA TYA A N N R TA R GC Y T N Y N N A T T A T A T A T A T A T A T GC GC

FIG. 7.2 Cont.

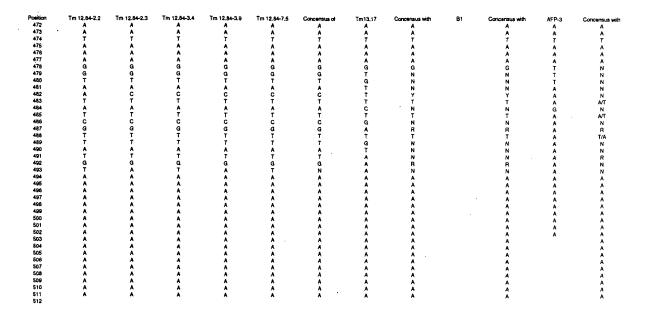


FIG. 7.2 Cont.

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FIG. 7.3 Cont.

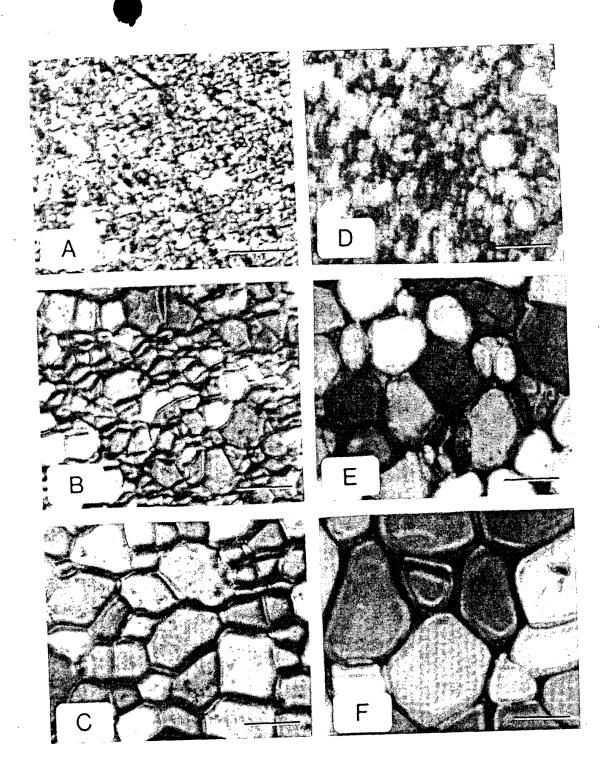


FIG. 8.0

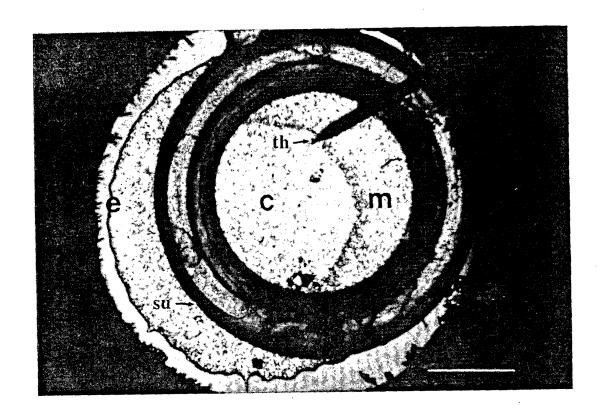


FIG. 8.1a

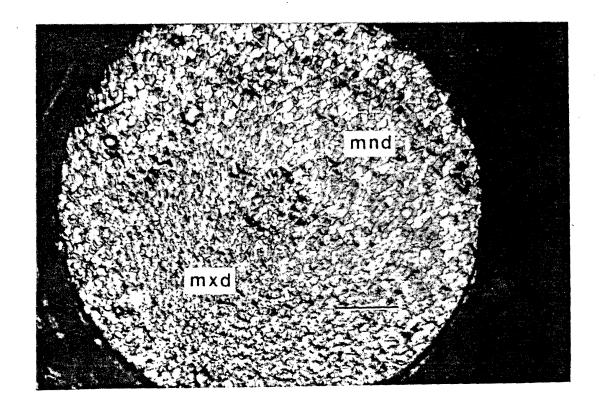
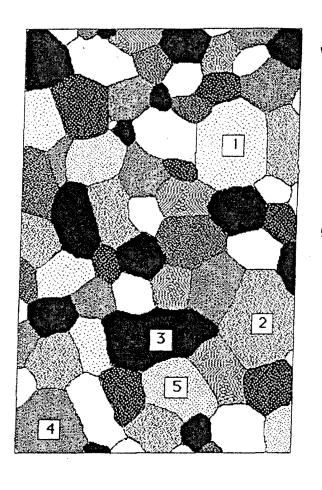
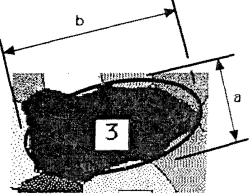


FIG. 8.1b





grain area=0.25mab

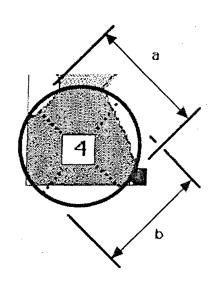


FIG. 8.2

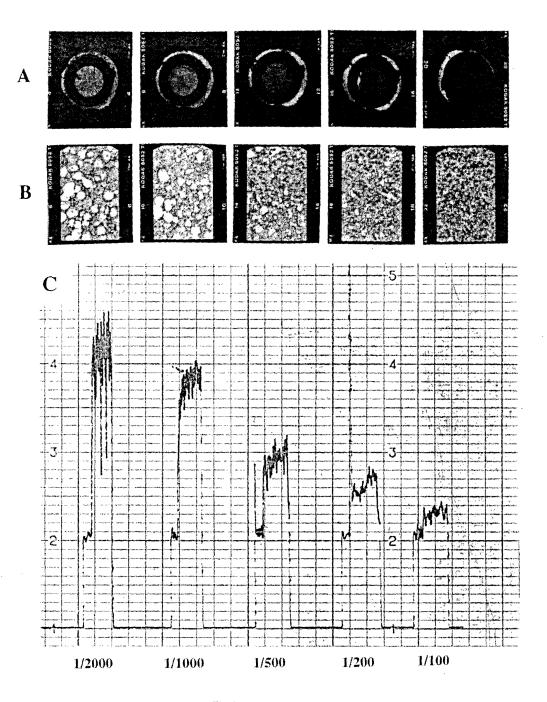


FIG. 8.3

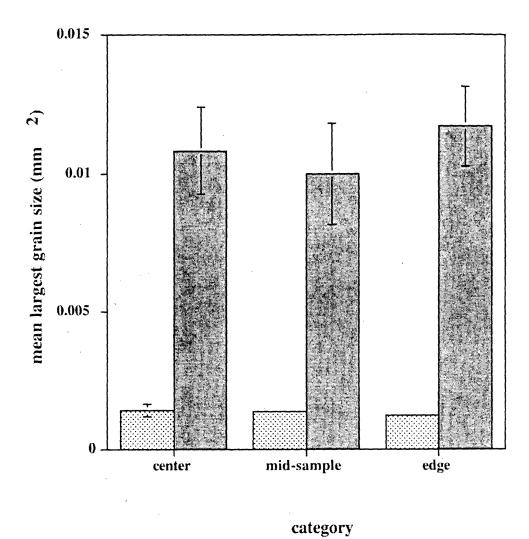


FIG. 8.4a

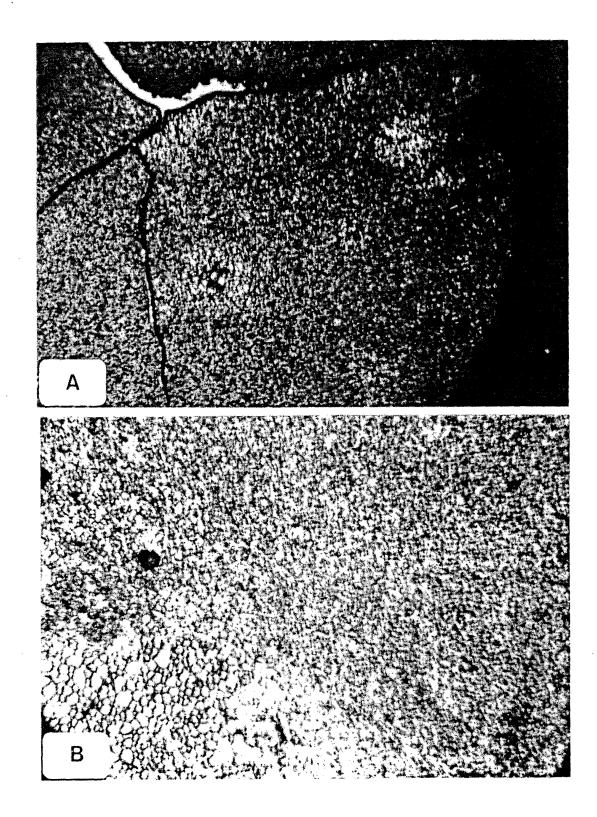


FIG. 8.4b

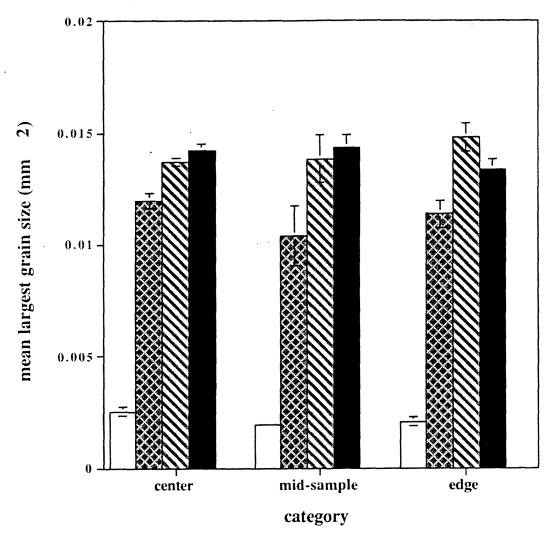


FIG. 8.5a

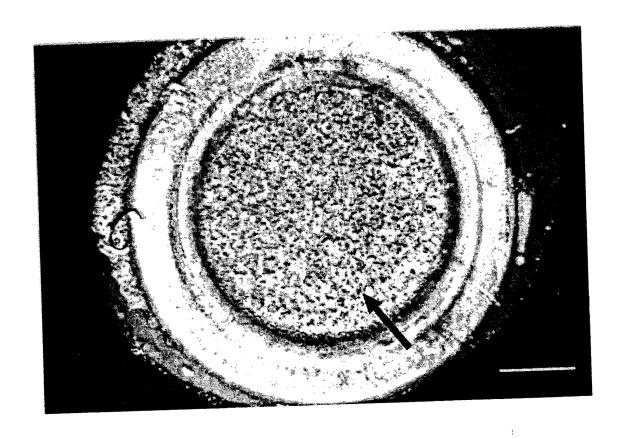


FIG. 8.5b

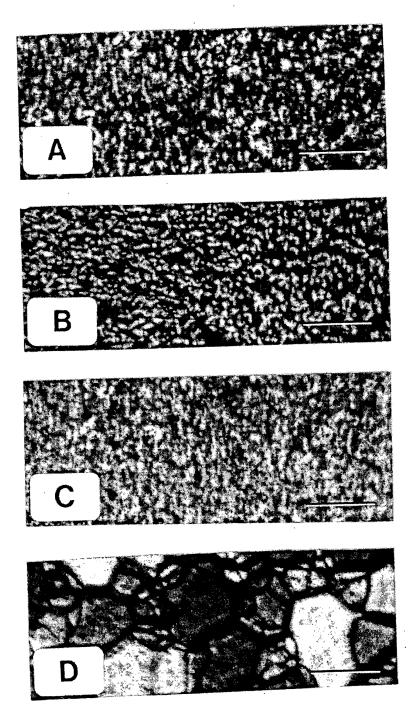


FIG. 8.6

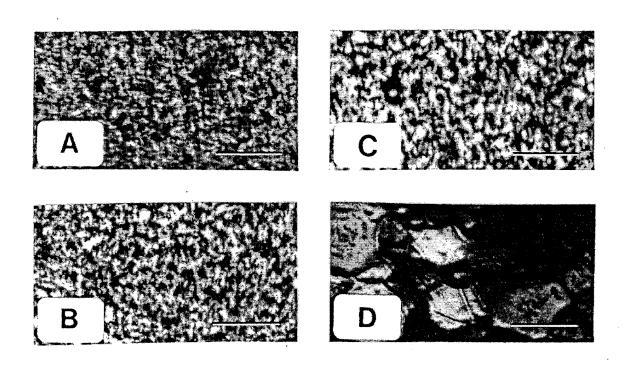
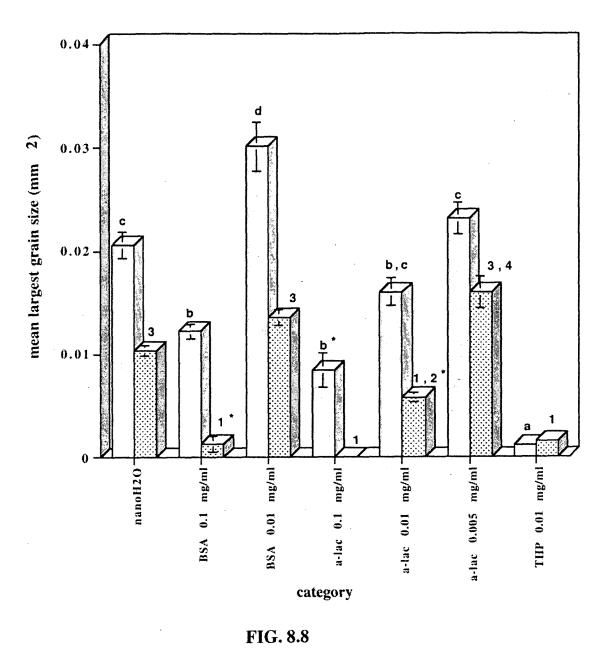


FIG. 8.7



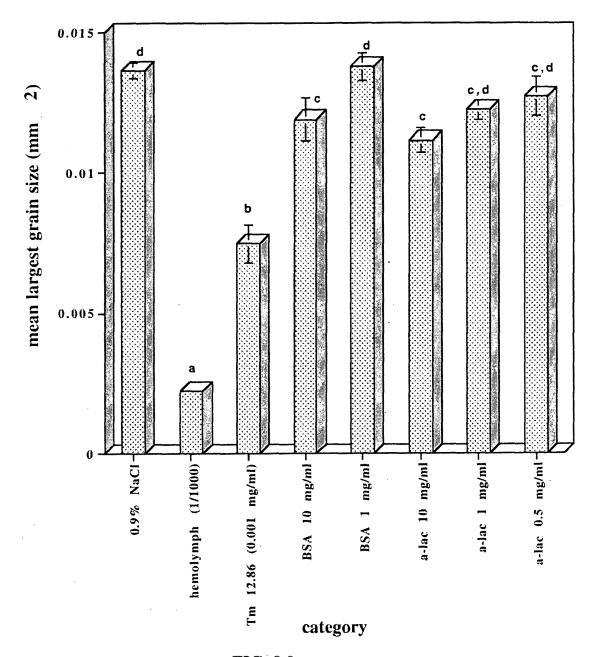
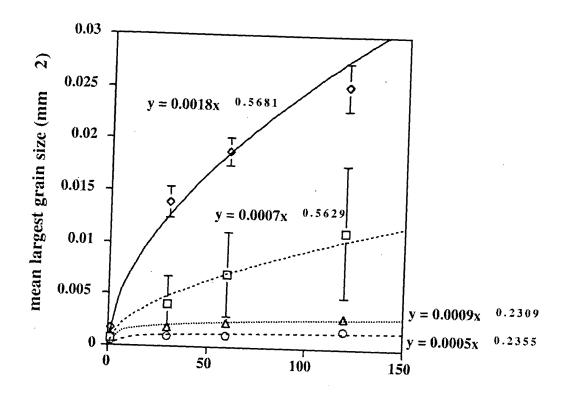


FIG. 8.9



time (minutes)

FIG. 8.10

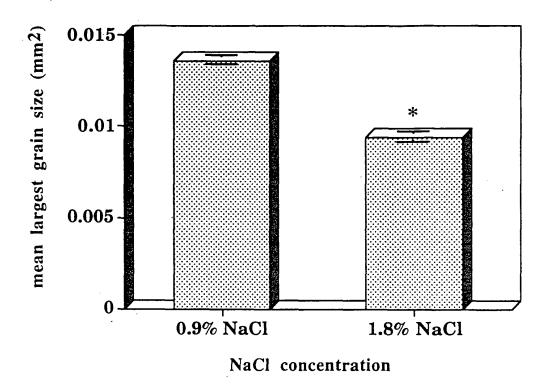
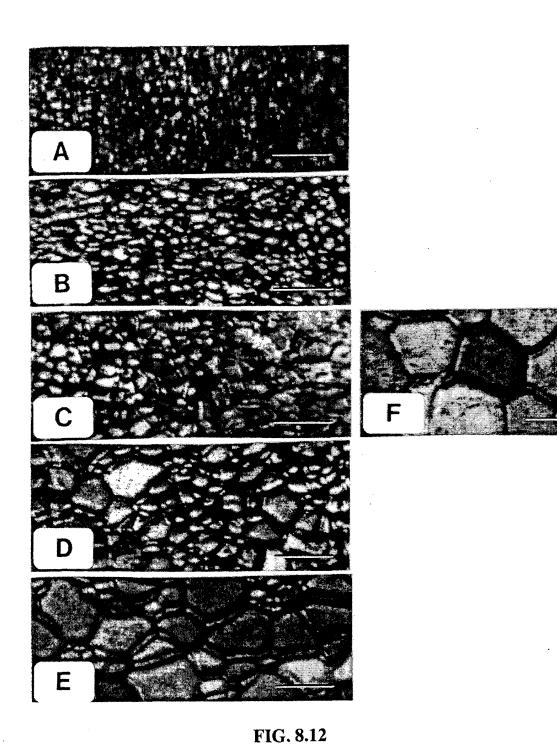


FIG. 8.11



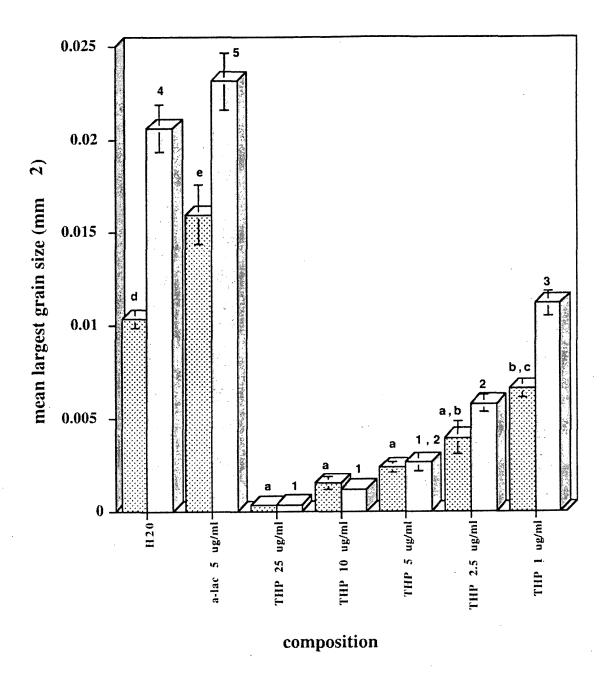


FIG. 8.13

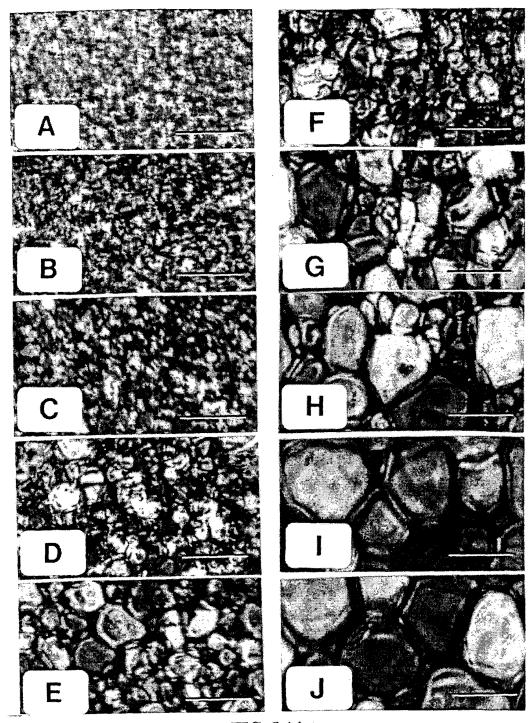
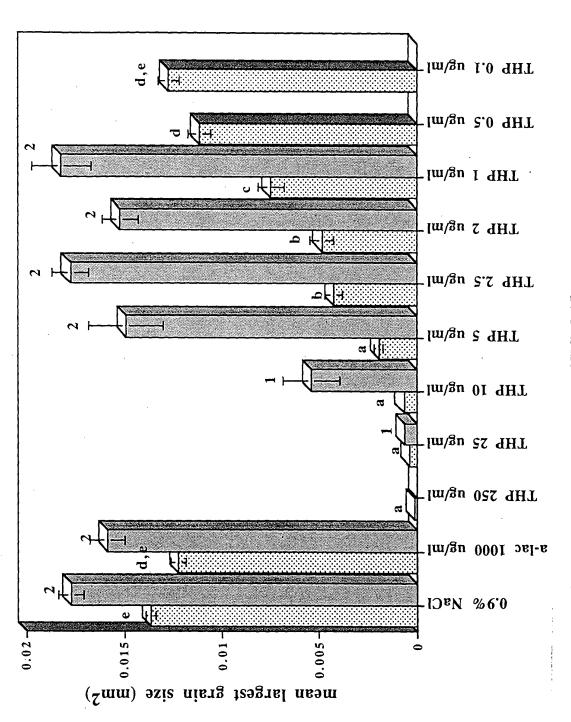


FIG. 8.14



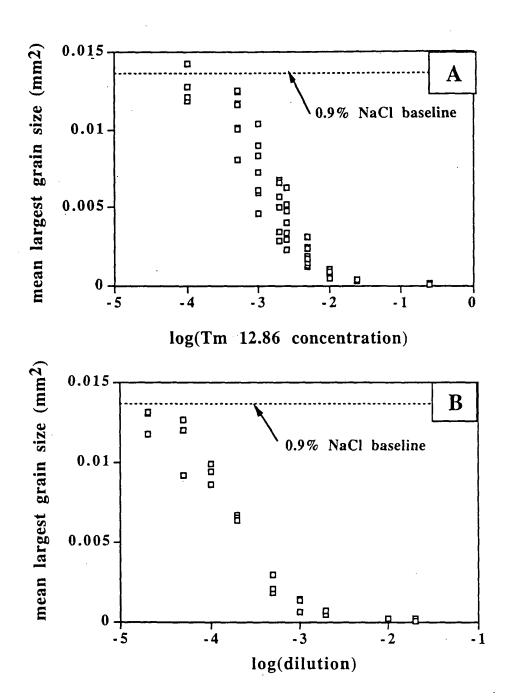


FIG. 8.16

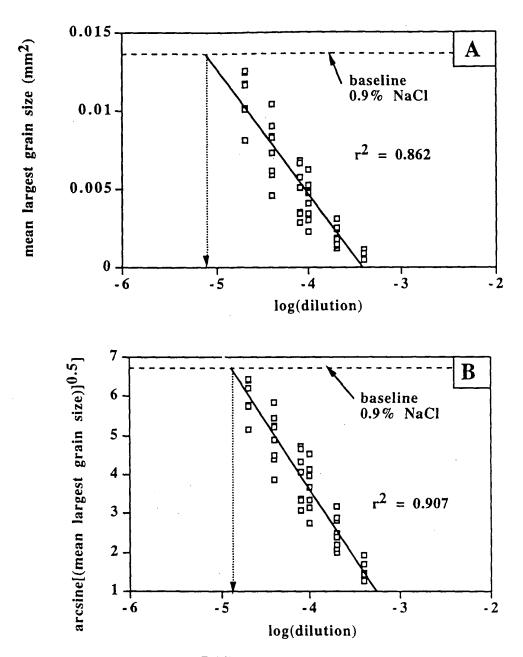
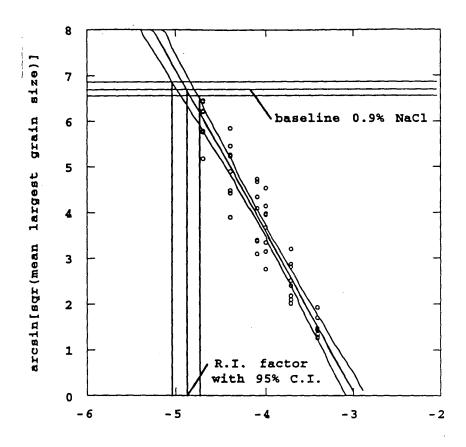


FIG. 8.17

.



log(dilution)

FIG. 8.18

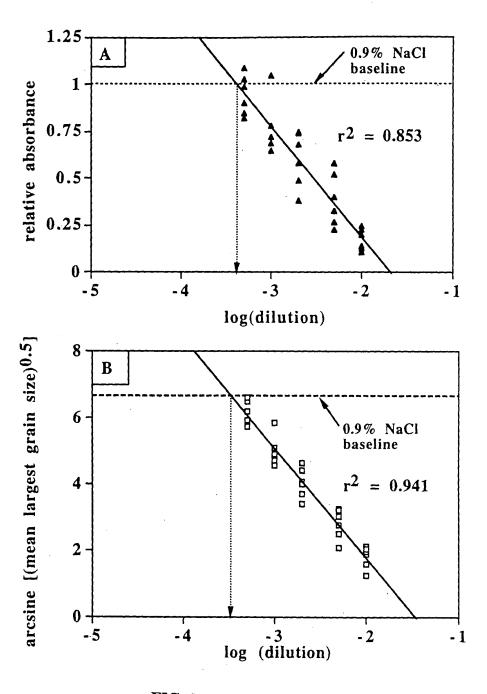


FIG. 8.19

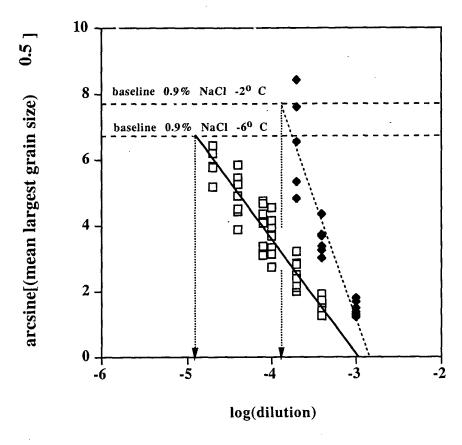


FIG. 8.20

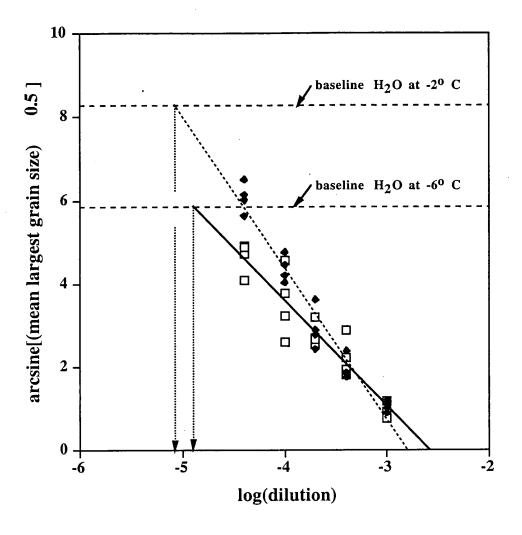


FIG. 8.21

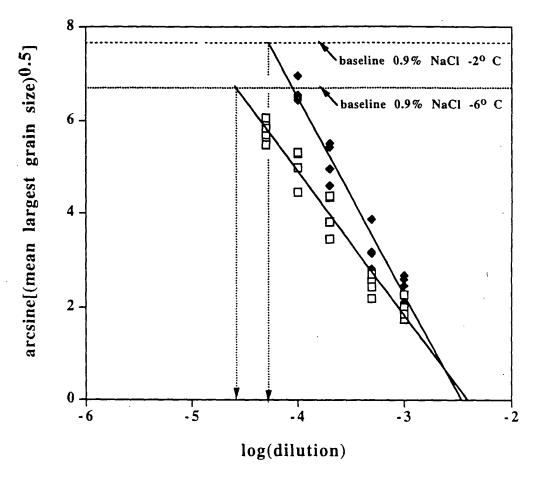


FIG. 8.22

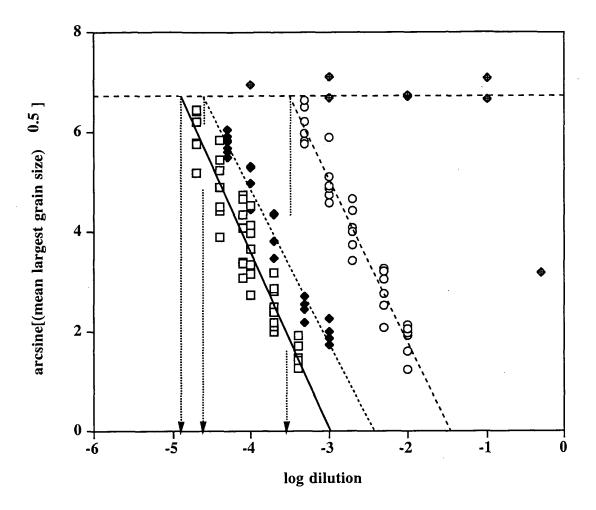
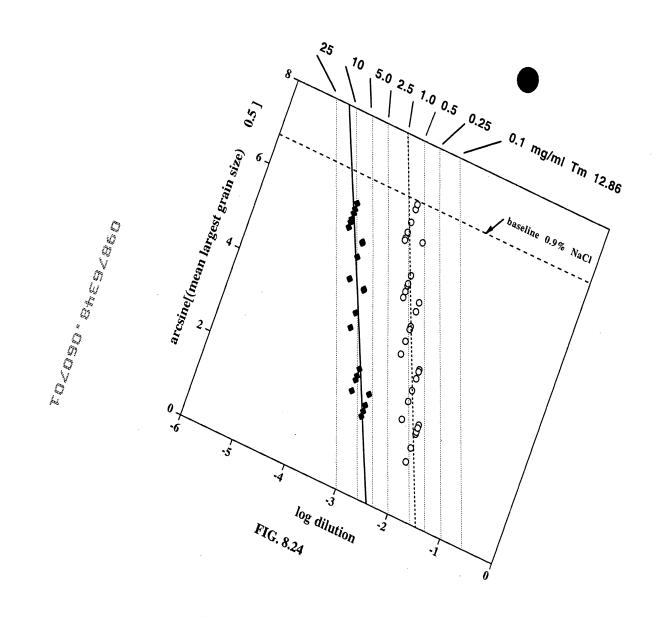


FIG. 8.23



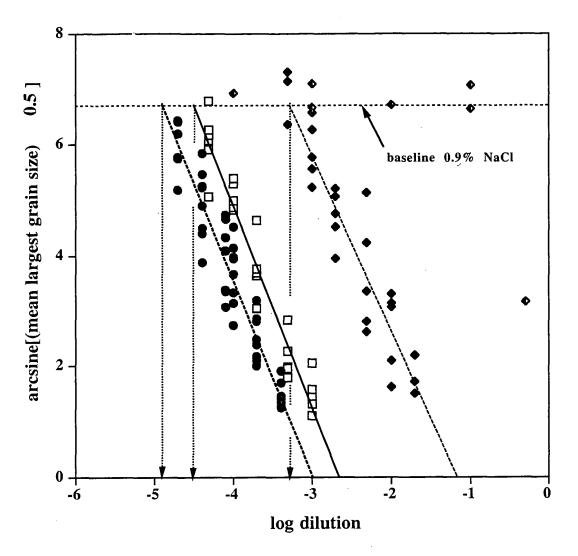
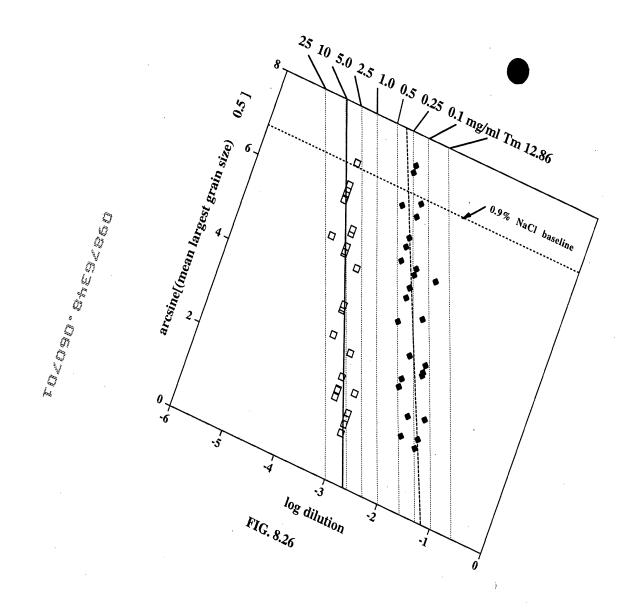


FIG. 8.25



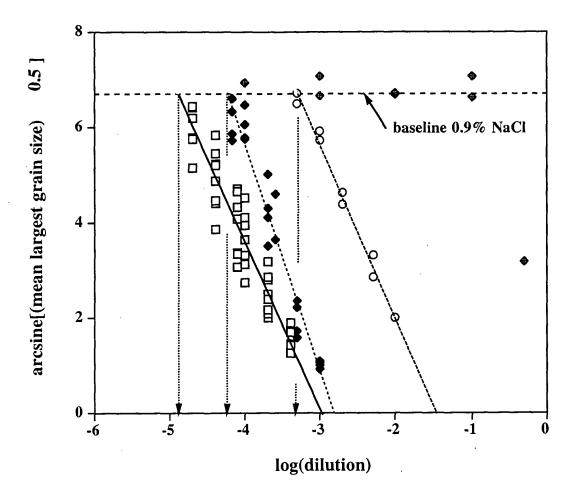


FIG. 8.27

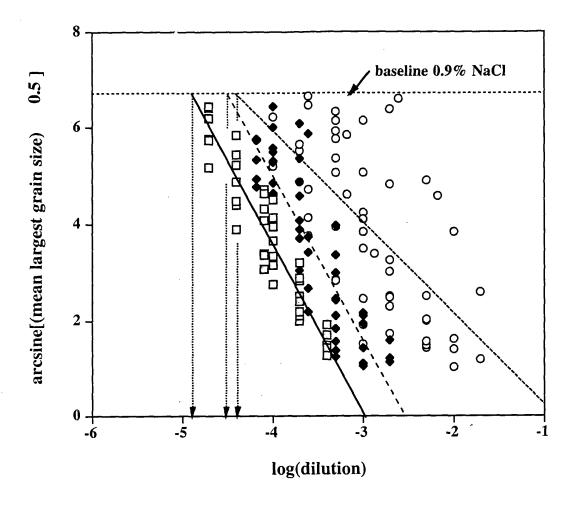


FIG. 8.28

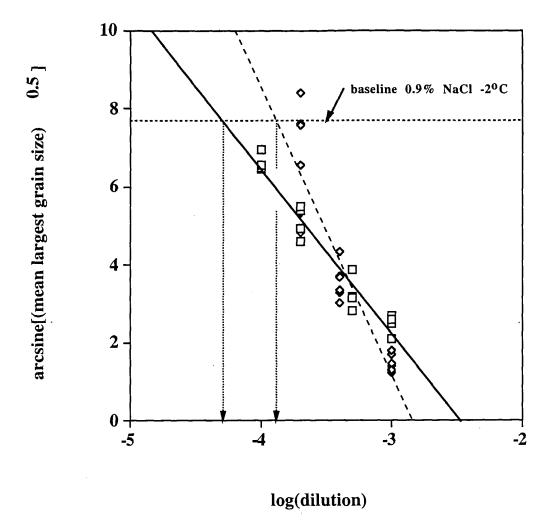


FIG. 8.29

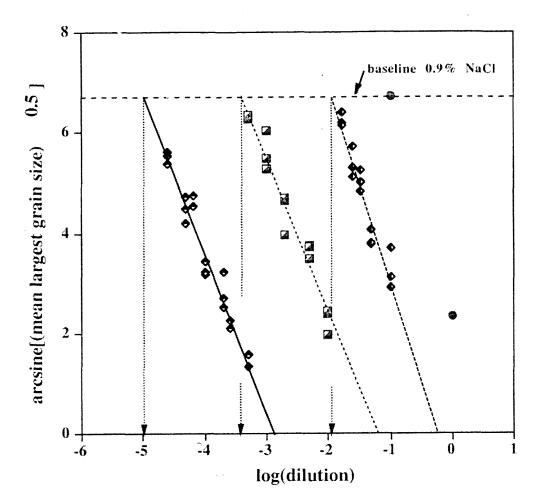


FIG. 8.30

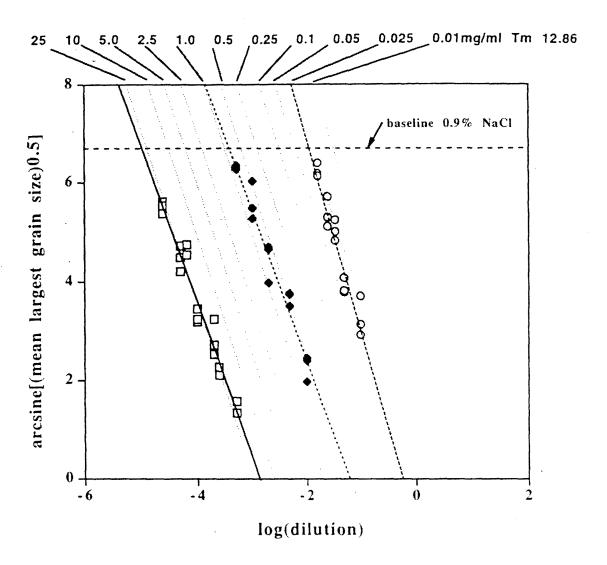


FIG. 8.31

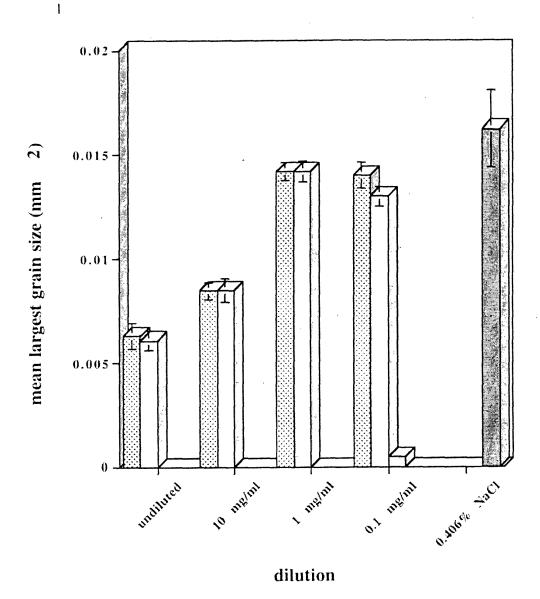


FIG. 8.32

1

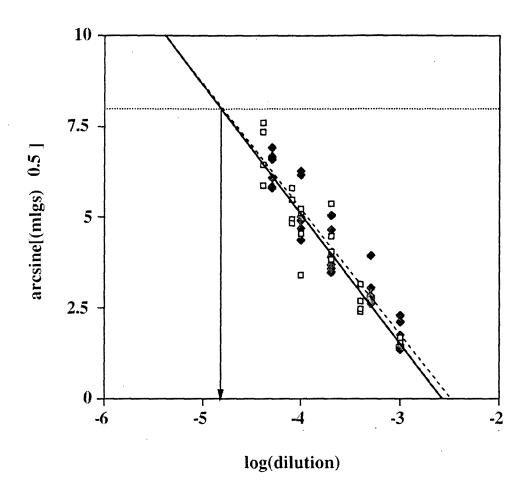


FIG. 8.33

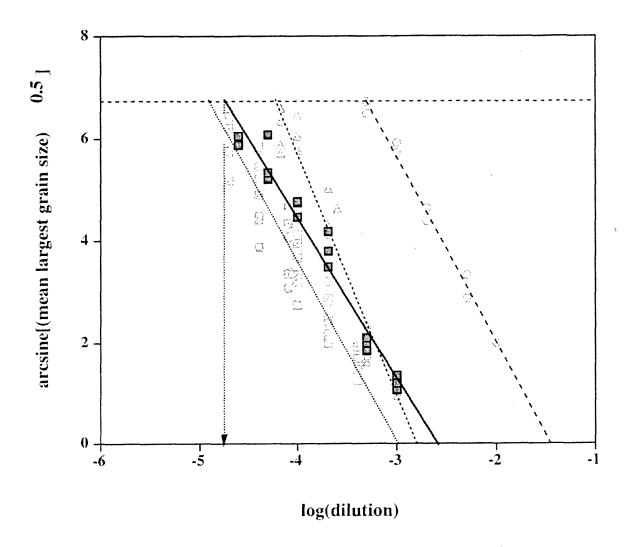


FIG. 8.34

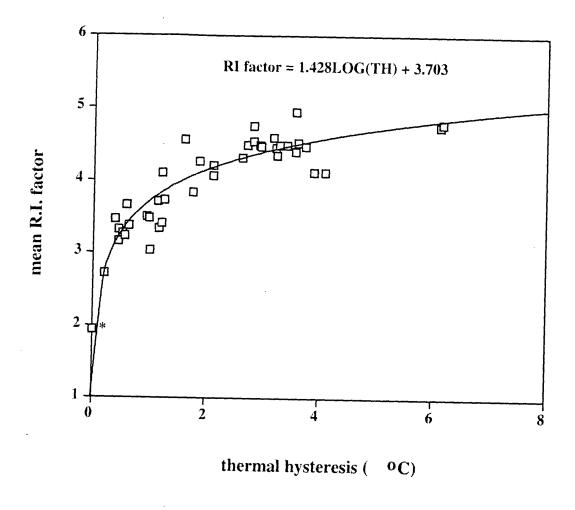


FIG. 8.35

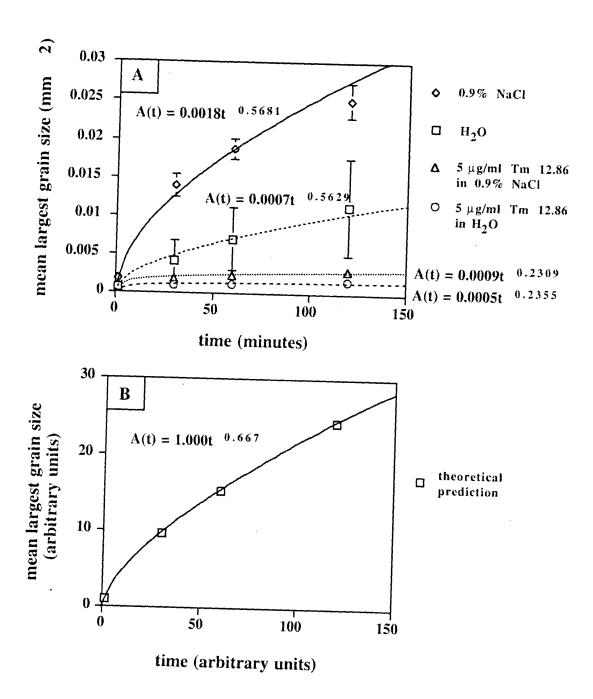
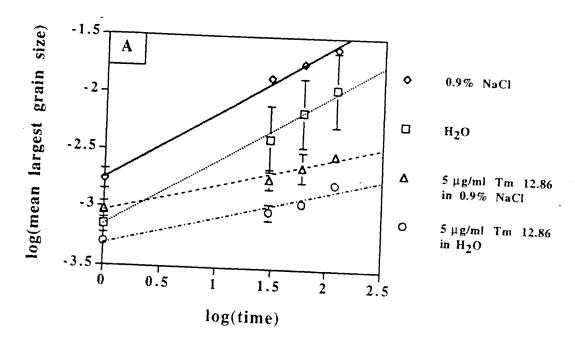


FIG. 8.36



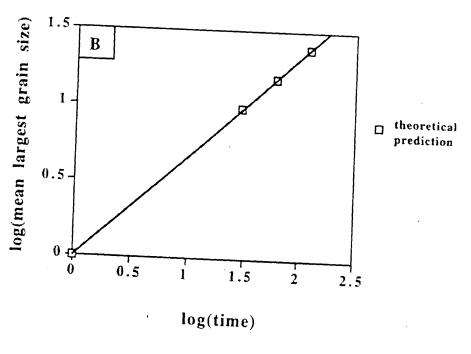
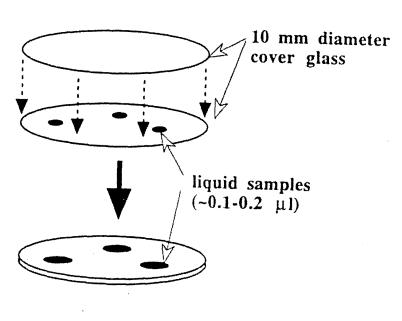


FIG. 8.37

"Sandwich" method of R.I. assessment

1.

2.





3. FREEZE ON ~-80 C ALUMINUM PLATE (~10 MIN.)



4. PLACE ON COLD STAGE, ANNEAL AT -6 C UP TO 12+ HOURS

FIG. 8.38

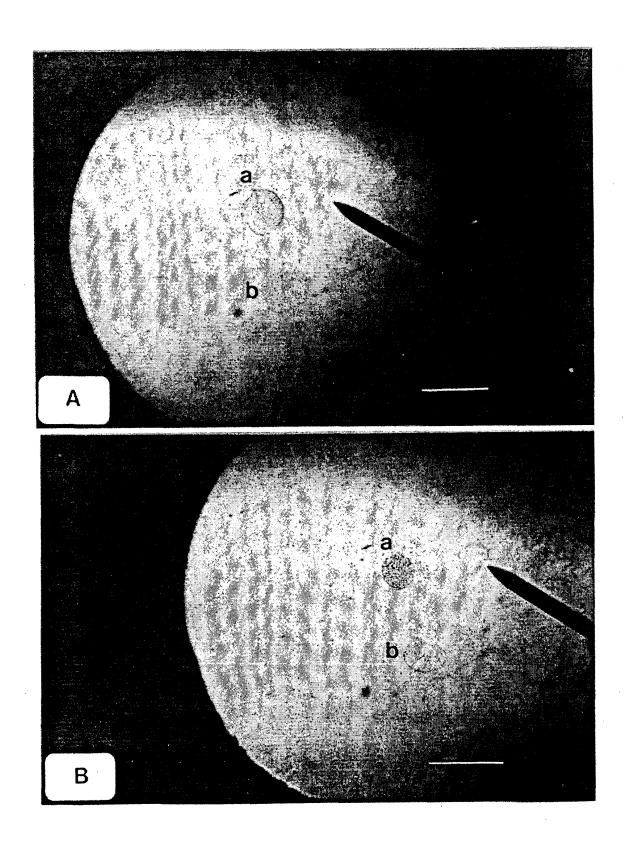


FIG. 8.39

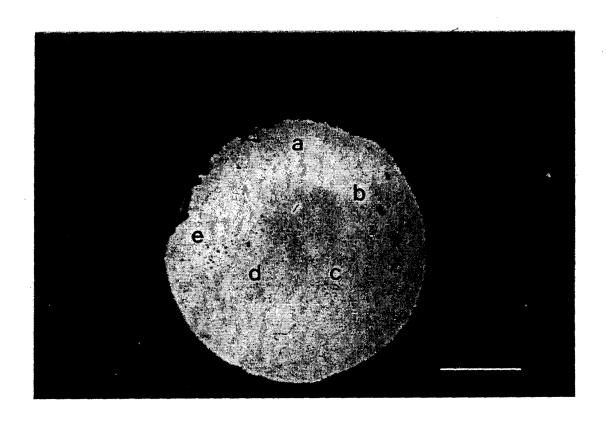


FIG. 8.40

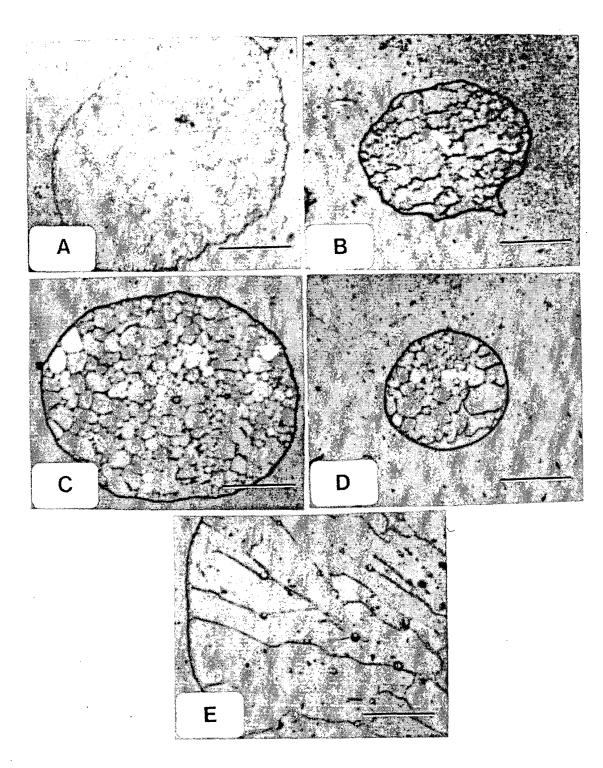


FIG. 8.41

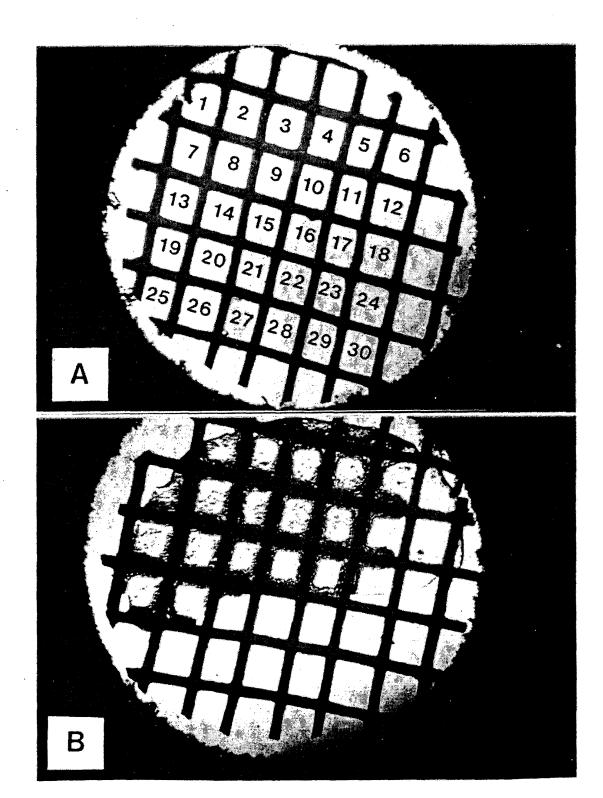


FIG. 8.42

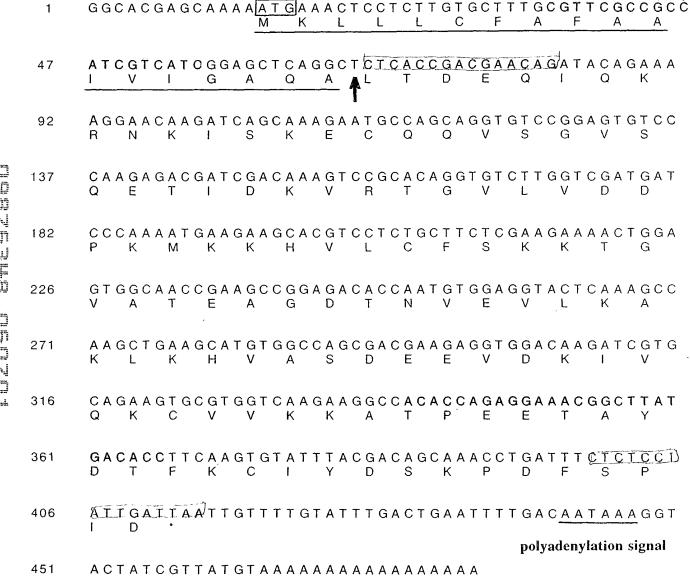
DNA sequence of Tm 13.17 cDNA clone

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61	CCCI	'CAT	TCT	GTT	GGT	CAC	ÁGT	TCA	\GGC	CCI	'GAC	CGA	.GGC	ACA	AAT	TGA	.GAA	ACT	GAA	CÁ
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	-						:			↑										
121	AGAT	'CAG	CAA	AAA	ATG	TCA	AAA	TGA	AAC	TGG	AĞT	GTC	GCA	AGA	GAT	CAT	AAC	CAA	AGC	TC
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181 GCAACGGTGACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTTTGCG												CGT	GGC	CAG	GA					
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241	ACGC	CGG	тст	GGC	CAC	GGA	АТС	GGG	AGA	GGI	GGT	GGT	CGA	CGT	GTT	GAG	GGA	GAA.	GGI	'GA
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421	AGTT	-				'1"I'G	AAC	CAC	CAC	GAC	TAG	TAG	ATG	GTT	CAA	ATG	rG.T.G	rTGC	.1.1.1	AC
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- 537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTTCCACCC



poly (A) tail

FIG. 8.44